THE FUNCTIONAL DISSECTION OF MOTION PROCESSING PATHWAYS IN THE HUMAN VISUAL CORTEX USING FMRI-GUIDED TMS

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Abstract

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Keywords: TMS, V5/MT, fMRI, function, TO-1, TO-2, motion perception, V3A

Motion-selectivity in human visual cortex comprises a number of different cortical loci including V1, V2, V3A, V3B, hV5/MT+ and V6 (Wandell et al., 2007). This thesis sought to investigate the specific functions of V3A and sub-divisions of hV5/MT+ (TO-1 and TO-2) by using transcranial magnetic stimulation (TMS) to transiently disrupt cortical activations within these areas during psychophysical tasks of motion perception. The tasks were chosen to coincide with previous non-human primate and human neuroimaging literature; translational, radial and rotational direction discrimination tasks and identification of the position of a focus of expansion. These results assert that TO-1 and TO-2 are functionally distinct subdivisions of hV5/MT+, as we have shown that both TO-1 and TO-2 are responsible for processing translational motion direction whilst only TO-2 is responsible for processing radial motion direction. In ipsilateral space, it was found that TO-1 and TO-2 both contribute to the processing of ipsilateral translational motion. Taken in a wider context, further results also suggested that these areas may form part of a network of cortical areas contributing to perception of self-motion (heading/egomotion), as TO-2 was not found to be responsible for processing the position of the central focus of expansion (imperative for self-direction). Instead, area V3A has been implicated as functionally responsible for processing this attribute of vision. Overall it is clear that TO-1, TO-2 and V3A have specific, distinct functions that contribute towards both parallel and serial motion processing pathways within the human brain.

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Chapter 1
Introduction

1.1 Overview

In order to perceive the visual world appropriately it is important to be able to process the motion qualities within a scene i.e. the speed, direction, type of motion of an object. This conveys the impression of an extremely complicated physiological demand, and yet from real-world experience it is known that visual motion processing is associated with an automatic, unconscious process. This is possible because neural activity related to visual motion is undertaken by several areas within the human cerebral cortex (see Figure 1.1), including but not restricted to, V1, V2, V3A, V3B, V5/MT+, V6 (Watson et al., 1993; Dupont et al., 1994; Tootell et al., 1995; Dupont et al., 1997; McKeefry et al., 1997; Smith et al., 1998; Culham et al., 2001; Pitzalis et al., 2010). Of these regions, one of particular interest and relevance is human area V5/MT+ (hV5/MT+) which is currently the area most frequently associated with motion perception (Zeki et al., 1991; Dumoulin et al., 2000).

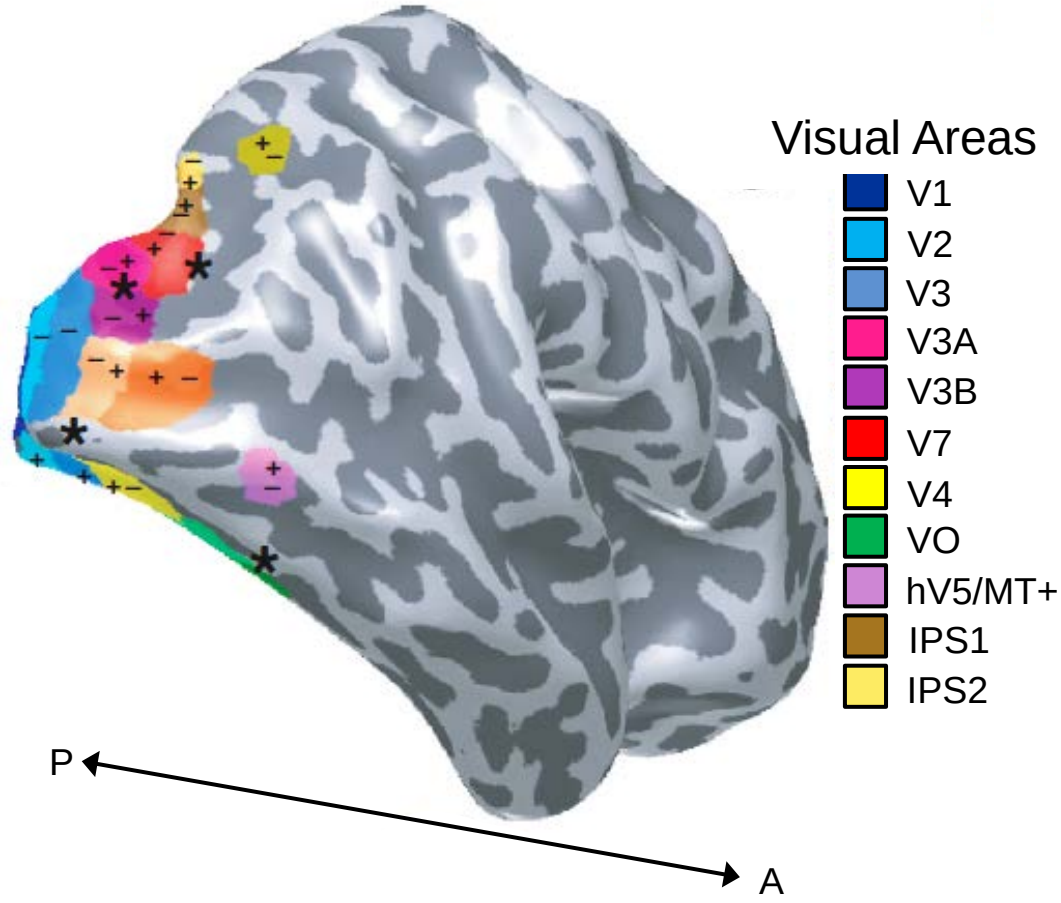

Figure 1.1. Figure showing location of visual areas on inflated right hemisphere (A = anterior; P = posterior). Dark grey patches indicate sulci, whilst light grey represents gyri. Superior visual field representations (+), inferior visual field representations (-), and foveal confluences (*) are shown for each area. (Adapted from Wandell et al., 2009).

The first attempts at understanding this visual area stemmed from a well-established understanding of the homologous area in the non-human primate brain: middle temporal area MT+ (Maunsell and Van Essen, 1987). This area is a complex, meaning it contains several smaller sub-divisions, each functionally responsible for processing different aspects of the moving visual scene. Within the monkey, these sub-divisions include MT, MST, FST and V4t (Desimone and Ungerleider, 1986; Komatsu and Wurtz, 1988; Tanaka et al., 1993; Nelissen et al., 2006; Kolster et al., 2009; Albright, 1984; Albright,

2012). As an example of a functional difference between them, neurons in area MT are referred to as generalised motion processors because they respond best to several different kinds of motion stimulus, whereas neurons in MST show more specific preferential response to optic flow and rotational stimuli (Saito et al., 1986; Mikami et al., 1986; Duffy and Wurtz, 1991a; Duffy and Wurtz, 1991b; Lagae et al., 1994).

In the human brain, hV5/MT+ is also a complex containing sub-divisions, but these sub-divisions are less well understood. So far the most reliable method for sub-dividing hV5/MT+ relies on an understanding of the receptive field (RF) size of neurons. Extrapolating knowledge from non-human primate research showing anterior MST contains larger RFs than posterior MT (Duffy and Wurtz, 1991a; Duffy and Wurtz, 1991b; Raiguel et al., 1997), human neuroimaging experiments have managed to show that RFs within hV5/MT+ follow a similar pattern of size change. This has allowed a consistent parcellation of this area into two distinct regions; TO-1 (posterior) and TO-2 (anterior) (Dukelow et al., 2001; Huk et al., 2002; Amano et al., 2009).

The next empirical question to answer is whether these sub-divisions are functionally distinct and if so, do their independent functions align with the known functions of the potentially homologous areas within the non-human primate brain. Previous neuroimaging experiments in humans have suggested that the functional specificity of these areas may be similar to those in non-human primates by showing that preferential increases in blood-oxygen-level dependent (BOLD) signal differ between TO-1 and TO-2 depending on the type of motion stimulus (Morrone et al., 2000; Kourtzi et al., 2002; Smith et al., 2006; Wall et al., 2008). However, these results are

not entirely consistent with primate literature as they lack an explicit functional distinction, and as they are based on neuroimaging data, they are purely correlative measures of cortical function. This means establishing causal links between motion perception and TO-1 and TO-2 is not possible using this data. In order to provide this information, it is necessary to use a technique for directly assessing the functionality of these areas. This can be achieved using transcranial magnetic stimulation (TMS).

TMS is a technique that allows transient and targeted disruption of normal cortical function, which is particularly useful for studying types of motion perception (Beckers and Homberg, 1992; Hotson et al., 1994; Beckers and Zeki, 1995; Anand et al., 1998; Walsh et al., 1998; Walsh and Cowey, 2000; Pascual-Leone and Walsh, 2001; Cowey et al., 2006; Laycock et al., 2007).

The experiments described in this thesis sought to actively disrupt normal cortical function of TO-1 and TO-2 following functional identification using fMRI. This disruption was measured across a series of behavioural tasks designed to investigate differences between the two target areas, and also to compare activity with that of a non-human primate.

1.2 Motion Processing Pathway

In humans, the visual cortex makes up approximately 20% of the overall cerebral cortex and comprises of approximately 4-6 billion neurons (Wandell et al., 2007). This large amount of cortex encompasses the entire occipital lobe and includes several regions of the neighbouring parietal and temporal lobes. However these regions are not all responsible for processing the

same properties of the visual scene. Instead, the visual cortex appears to be sub-divided into smaller areas that are specialised for specific attributes of visual processing (Zeki et al., 1991). In this respect, the visual cortex can be considered as containing several functionally-defined, highly specialised regions of cortex. These sub-regions can be distinguished and identified through several methods. Two commonly used methods of discriminating between visual areas involve the use of neuroimaging and include: functional localisers and retinotopic mapping.

Visual processing pathways tend to be organised into hierarchies, usually beginning at the more generalised primary visual cortex and gradually progressing to more specialised areas that are better able to analyse specific attributes within a visual scene (Zeki et al., 1991). In this sense, lower visual areas compute and process the underlying features of a visual scene (low-level vision), whereas the higher extrastriate areas are typically responsible for more complex aspects (high-level vision) and also contribute to top-down processing (Felleman and Van Essen, 1991; Lee et al., 1998).

One such specialised hierarchy of particular interest for this thesis relates to the processing of visual motion. Perceiving, following, understanding and even predicting the movement of objects is an important component of visual perception in everyday life. It is not only important for understanding how objects are moving in space relative to the individual but it is also useful for being able to understand self-motion and heading information, and for guiding eye movements (Lappe et al., 1999; Britten, 2008).

Within the visual cortex there are two main pathways that contribute to processing of vision simultaneously. These pathways are referred to as 'dorsal' and 'ventral' parallel processing steams, and have been identified in both non-human primates (Mishkin and Ungerleider, 1982) and humans (Goodale and Milner, 1992). The dorsal stream is often correlated with perception of dynamic, spatiotemporal information and is generally processed through the parietal regions, whereas the ventral processing stream is correlated with processing static, spatial visual information more commonly associated with the temporal lobe (Figure 1.2). Therefore it is understood that the motion perception hierarchy forms part of the dorsal processing pathway.

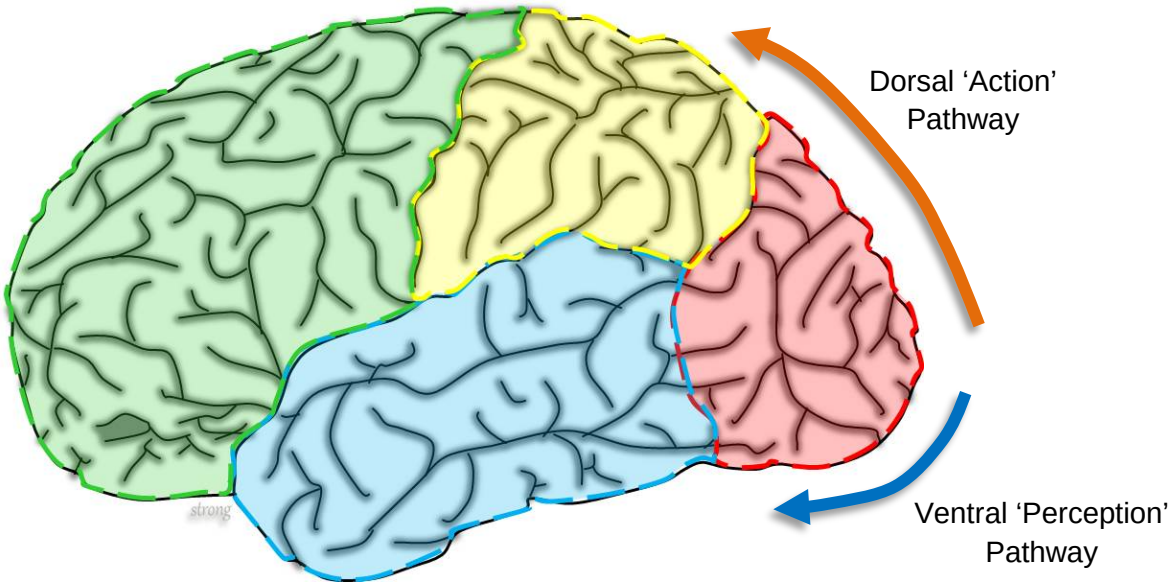

Figure 1.2. Schematic representation of dorsal and ventral pathways with colour co-ordinated segmentations of the four lobes. The dorsal stream progresses through the occipital lobe (red) to the parietal lobe (yellow), whereas the ventral stream progresses through the occipital lobe through to the temporal lobe (blue).

Historically, the most reliable and effective method of investigating motion processing within the brain was to use non-human primates as subjects due

to the advantages and precision of single cell recordings along with the limitation that when this type of research was first explored it was technologically impossible to examine the human brain *in-vivo*. However, as technology has improved over recent years, researchers have been able to shift this focus onto motion-processing within the human brain using a variety of methods: positron emission tomography (PET), functional magnetic resonance imaging (fMRI) and transcranial magnetic stimulation (TMS), for example. Despite the genetic similarities between primates and humans, it has been found that the particular visual areas involved in the motion hierarchy differ slightly between species, and the specific functional differences between the multiple motion areas in the human brain are still relatively unclear.

1.3 The Motion Hierarchy in Monkey and Man

In non-human primates, lesion, neuroimaging and electrophysiological studies have shown motion sensitive areas extend across a number of regions within the visual cortex through V1, V2, up into V3, and MT+ (Newsome and Paré, 1988; Rees et al., 2000; Liu and Newsome, 2005). Whereas human neuroimaging studies have shown that viewing moving stimuli will elicit an increase in cortical activity in V1, V2, V3A, V3B and hV5/MT+ (Tootell et al., 1995; Tootell et al., 1997; Smith et al., 1998). These studies have also shown that the increase in cortical activity associated with viewing moving stimuli is greater in the higher extrastriate areas V3B (KO), V3A and hV5/MT+ (Tootell et al., 1995). From this comparison it is clear that

although the hierarchies are similar, there are marked differences between species.

1.3.1 Monkey MT+

In non-human primates, MT+ is known to be a highly motion-sensitive complex and it is proposed to consist of five functional sub-divisions (Kolster et al., 2009). These sub-divisions include: the middle temporal area (MT), the dorsal region of the medial superior temporal area (MSTd), the lateral ventral region of the medial superior temporal area (MSTl/v), the fundus of the superior temporal area (FST), and V4 transitional zone (V4t) (see Figure 1.3).

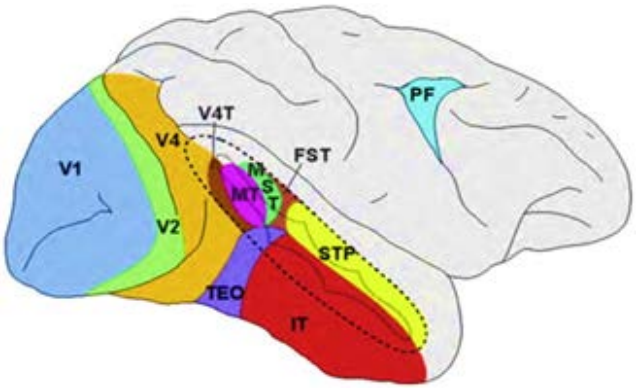

Figure 1.3. Lateral view of monkey cortex with partially unfolded superior temporal sulcus (STS) shown within black dotted lines. Motion areas (MT, MST, FST and V4t) are shown in differing coloured neighbouring regions within this zone. Other areas shown include: STP (superior temporal polysensory area), TEO (posterior temporal), IT (inferior temporal), and PF (prefrontal). (Adapted from Albright, 2012).

Several electrophysiology and neuroimaging experiments have successfully shown that these five areas have different neuronal properties and are

responsible for processing different aspects of visual motion. However, in this thesis the focus will remain on areas MT, MSTd and MSTl/v.

The most posterior sub-region, monkey area MT (see Figure 1.3), is reported to have smaller receptive fields (RFs) than more anterior areas within this complex, and it therefore demonstrates a slightly clearer retinotopic map (Gattass and Gross, 1981; Albright and Desimone, 1987; Maunsell and Van Essen, 1987). This permits an understanding of a retinotopic organisation for this area, as it is possible to study firing rates of neurons with positional preferences. Studying RFs within MT has also identified a receptive field bias, as neurons within this region seem to over-represent the inferior contralateral quadrant relative to the superior contralateral quadrant (Maunsell and Van Essen, 1987).

When investigating motion sensitive areas in the monkey brain, single cell recording is often employed in order to ascertain whether cells are direction selective. This categorisation applies to any neurons found to show a greater increase in activity for a restricted, preferred range of directions. It has been reported that between 70-100% of cells within MT are directionally selective (Dubner and Zeki, 1971; Albright, 1984) which provides further evidence for this area being important for processing movement.

Functionally, MT is motion selective, but it appears to demonstrate a less specialised type of activation than the more anterior sub-regions of MT+ because cells within this area respond to a larger range of moving stimuli (Lagae et al., 1994). In accordance with this, lesion studies have demonstrated the importance of MT for the perception of direction as it has

been found that lesioning MT significantly increases thresholds for direction discrimination without affecting contrast discrimination ability (Newsome and Paré, 1988). As the discrimination deficit is restricted specifically to a direction task, this study is considered evidence for motion-selective function within MT. Contributing to the idea that MT responses are broadly tuned for motion, these researchers also concluded that visual area MT should be viewed functionally as a 'general purpose' motion processor (Newsome and Paré, 1988). Further electrophysiological experiments have demonstrated that in addition to this direction selectivity, neurons within macaque MT are clustered according to the preferred speed of the individual neurons (Liu and Newsome, 2003). This suggests that area MT should be important for the perception and processing of both direction and speed, which has been reinforced by findings from a neuroimaging experiment (Orban et al., 2003). This study not only found preference for speed and direction within MT, but also found increased activation during the perception of moving translational dots and in the perception of 3D structure from motion.

In addition to this functional specialisation, another key role of MT is the involvement in both feed-back and feed-forward mechanisms (Felleman and Van Essen, 1991; Pascual-Leone and Walsh, 2001). This means that whilst MT receives afferent signals from lower visual areas (V1, V2, etc), it also receives efferent signals from nearby higher motion sensitive areas (MSTd, MSTl/v, etc). This is an important factor because perceptual decisions are not only influenced by incoming signals, but also by analysis from higher cortical areas (Ungerleider and Desimone, 1986). This leads on to visual area MST.

As a whole, the antero-lateral portion of MT+ in the macaque monkey (MST) receives most of its cortical input from MT and it is therefore no surprise that like MT, it is also strongly affiliated with the processing of motion (Maunsell and van Essen, 1983; Ungerleider and Desimone, 1986). MST is consistently reported to have larger receptive fields than MT (Duffy and Wurtz, 1991a; Duffy and Wurtz, 1991b; Van Essen et al., 1981; Raiguel et al., 1997), which means it is difficult to accurately attribute a retinotopic organisation to these regions of cortex. However, in 2009, one lab managed to identify several retinotopic representations within this region by using a 7T MRI to show awake monkeys retinotopic ring and wedge stimuli. They managed to find evidence of contralateral maps within MSTd and MSTl/v that lie adjacent to the superior boundary within MT (Kolster et al., 2009).

Functionally, research investigating anaesthetised and awake monkeys has shown that cells in MST show greater activation for 3D rotation and expansion optic flow stimuli than to 2D-planar translational motion (Saito et al., 1986; Tanaka and Saito, 1989; Duffy and Wurtz, 1991a; Duffy and Wurtz, 1991b; Lagae et al., 1994). In particular, the dorsal portion of MST (MSTd) appears to be the most important area for processing and analysing radial and circular visual motion (Saito et al., 1986; Tanaka and Saito, 1989). Another proposed function of MST is detection and encoding of heading direction and self-motion. The reason for this proposal is due to findings suggesting that ~90% of neurons within this area show differential signal responses when the centre of motion is shifted away from the centre of the visual field, and that overall there is a bias for neurons to prefer centre of

motion that correlates with the central visual field (Duffy and Wurtz, 1995; Orban et al., 1995).

MST cells, despite being more specialised for certain patterns of movement, appear to be less particular about the size, speed or location of the stimulus, providing it falls within an appropriate receptive field (Andersen et al., 1990; Celebrini and Newsome, 1994). Though there is evidence of a slightly greater firing rate within certain MST cells when the stimulus is large (Andersen et al., 1990). Previous studies have also reported finding direction selective cells (D cells) within MST that respond to wide-field movement but not the movement of a single bar (Tanaka et al., 1986). These cells were labelled ‘field cells’ as they appeared to detect coherent movement of a large array of dots, whilst overlooking the motion of objects within the array.

It is theorised that the speciality for optic flow exhibited by MST neurons is due to a contribution to the processing of heading and self-motion; information which is required for moving through the visual world. Further evidence supporting this claim extends from reports that cortical neurons within MST feed-forward to the parietal association cortex, which is an area that combines both retinal and non-retinal positional information (Mesulam et al., 1977; Hyvärinen, 1982).

When considered as two separate regions, MSTd and MSTl/v, a few key differences emerge. For example, MSTd shows greater activation for large field motion than for small spots of motion, which is consistent with its previously reported preference for optic flow stimuli (Saito et al., 1986). In contrast to this, neurons in MSTl/v prefer small, localised spots of light and

are likely to be involved in maintenance of pursuit eye movements (Komatsu and Wurtz, 1988; Dursteler and Wurtz, 1988; Komatsu and Wurtz, 1989).

Overall, MST appears to be slightly more specialised than MT as it has specific preferences for optic flow stimuli within MSTd and visual pursuit within MSTl/v.

1.3.2 Human hV5/MT+

The main motion-sensitive area in the human visual cortex is located on the lateral surface of the dorsal/posterior limb of the inferior temporal sulcus (Dumoulin et al., 2000). This area is referred to as hV5/MT+ because it is assumed that this area is the homolog to macaque MT+ (see Figure 1.3) and therefore that it will similarly be a complex contain several smaller areas (Zeki et al., 1991; Culham et al., 2001). However the number and organisation of sub-regions within human hV5/MT+ is much less clearly understood than those present in monkey MT+. This is partly because very precise techniques such as single-cell recordings are not possible within human cortex *in vivo*, and also because the resolution of neuroimaging techniques has only recently become refined enough to produce results that are appropriate for anatomically adjacent, small cortical areas.

Of these potential sub-divisions within hV5/MT+, the most investigated motion processing region is area V5/MT, which is located posteriorly within the complex (Zeki et al., 1991). It is characteristic of other extra-striate regions in that neurons within it have large receptive fields, thereby making it difficult to measure retinotopic maps using fMRI (Wandell et al., 2007).

However, in spite of this difficulty, it is estimated that V5/MT contains a full representation of the contralateral hemifield, and because of the size of the receptive fields, this representation is estimated to extend ~15° into the ipsilateral hemifield (Tootell et al., 1995).

Over recent decades, neuroimaging and psychophysical studies have successfully associated area V5/MT with functions including but not restricted to: motion detection (Thakral and Slotnick, 2011), the perception of speed (McKeefry et al., 2008; McKeefry et al., 2010), motion imagery and implied motion (Goebel et al., 1998; Kourtzi and Kanwisher, 2000), and coherence of motion (Braddick et al., 2001).

The most obvious function for a motion-sensitive area in the brain is to be able to detect moving visual stimuli. Numerous fMRI experiments have shown increased activation in hV5/MT+ when moving visual stimuli are presented (Zeki et al., 1991; Chawla et al., 1998; Sunaert et al., 2000; Sack et al., 2006), and there have been numerous TMS experiments showing that when TMS is applied to hMT/V5+ motion processing is disrupted (Hotson and Anand, 1998; Walsh et al., 1998; Matthews et al., 2001; Pascual-Leone and Walsh, 2001; McKeefry et al., 2008; Burton et al., 2009; McKeefry et al., 2010). In particular, there is evidence to suggest that application of TMS to V5/MT disrupts ability of subjects to detect motion providing it falls within contralateral visual space (Thakral and Slotnick, 2011). This shows that V5/MT has a representation that, like earlier visual areas, is fairly restricted to the contralateral visual field and that area V5/MT is probably necessary for the perception of motion.

With regards to identification of other areas within the hV5/MT+ complex, the path towards distinguishing between them has proven to be technologically difficult and inconsistent. The standard method for distinguishing boundaries between areas is to use retinotopic mapping paradigms (Engel et al., 1997), but due to limitations arising from the spatial resolution of fMRI procedures and the increasingly large receptive field sizes of neurons within these visual areas, this technique does not reliably confirm existence of these sub-divisions. This occurs because when attempting to retinotopically map the visual field using the standard checkerboard rings and wedges, the neurons with larger RFs exhibit activation that does not change appropriately as a function of time. This means that the representation of the map will be inaccurate and incomplete (Figure 1.4). Furthermore, some areas may not exhibit a map at all, which will cause them to be wrongly identified as non-responsive cortex (Wandell et al., 2007). Another limitation of this technique occurs if the neuronal RFs happen to be representing the central, foveal region of visual space. This leads to a consistent level of activation being produced in these areas throughout the rotating wedge stimulus presentation. The resulting lack of polar angle representation will make it difficult to distinguish accurately between these motion sensitive visual areas (Dumoulin and Wandell, 2008; Schira et al., 2009).

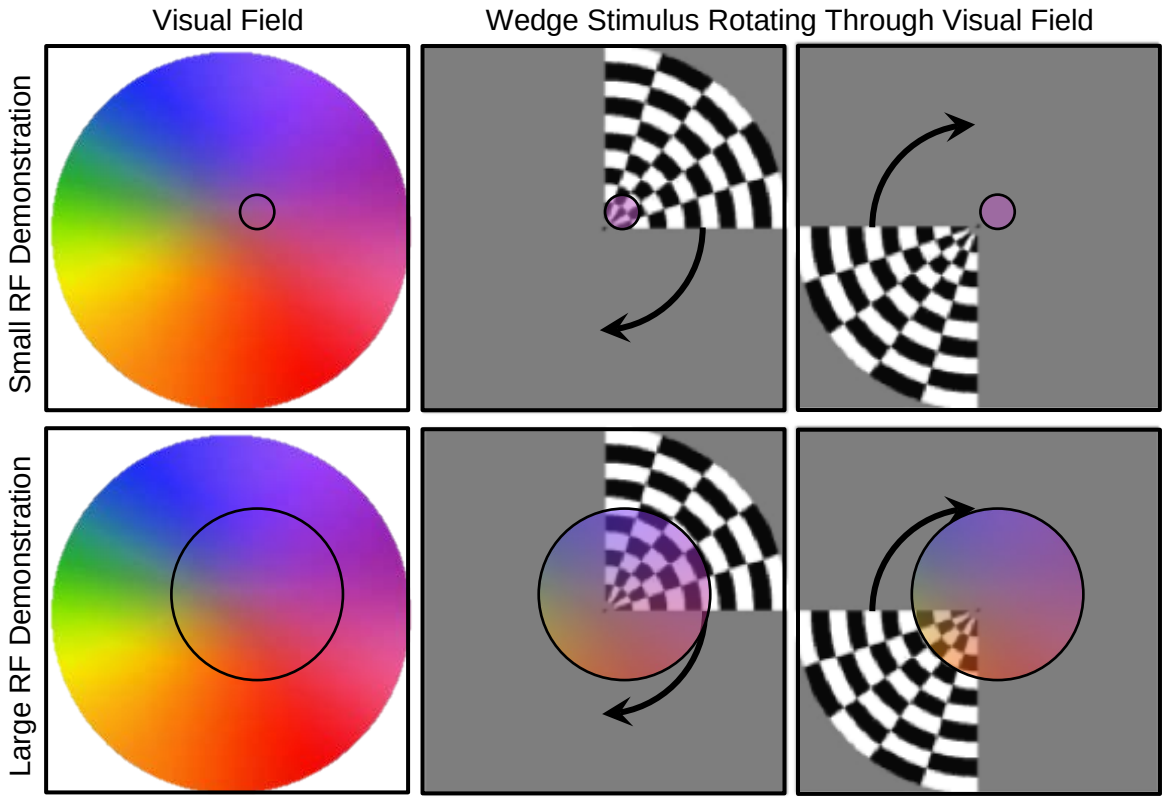

Figure 1.4. Figure demonstrating potential for error when retinotopically mapping areas with large receptive field (RF) sizes. The left column shows a colour-coded representation of the visual field (VF), whilst the middle and right columns shows the checkerboard wedge stimulus used to map this part of the VF. The top row highlights the success of this technique for small RFs, as it is only activated by one wedge position. The bottom row shows the limitation of this technique for larger RFs as this RF would be activated by all rotations of the wedge.

1.3.3 Recent Developments in Understanding of hV5/MT+

The issues highlighted above regarding the ability to measure retinotopic maps within the hV5/MT+ sub-divisions inspired some researchers to devise new means of differentiating between them. Recent research has consistently managed to demonstrate that by utilising appropriately designed neuroimaging techniques, it is possible to sub-divide this motion sensitive

area into at least two (potentially more) satellite regions (Dukelow et al., 2001; Huk et al., 2002; Amano et al., 2009; Kolster et al., 2010).

Initial methods for distinguishing between putative MT and MST within the human visual cortex involved taking advantage of the known properties of neurons within these areas. In this instance, it is well documented that the receptive field size of neurons are large within macaque MT and MST, with the largest receptive fields located in anterior MST. It is even reported that in monkeys, the receptive fields for MST can extend up to 40° within the ipsilateral visual field (Raiguel et al., 1997). This suggests that if it were possible to identify any clusters of neurons within human hV5/MT+ that were activated by ipsilateral stimulation; it would likely correlate with this anterior area.

The ipsilateral visual field coverage by anterior MST allowed Dukelow et al. (2001) to create visual stimuli that would limit activation to the ipsilateral MST region in human visual cortex. They presented moving visual stimuli (white dots on a black background) between 15-45° within the periphery of the visual field in the hopes of activating this ipsilateral MST region without activating the posterior MT. It was hypothesised to be successful as neurons in MT are not predicted to extend as far within the ipsilateral visual field. Data recorded from this experiment reinforced this distinction as it was found that they were able to limit any ipsilateral cortical activation to only an anterior parcel of the hV5/MT+ complex. If tested in both the left and right visual field, this anterior parcel could then be subtracted from the larger contralateral hV5/MT+ activation in order to localise and distinguish between the two areas (Figure 1.5). On average, Dukelow et al. reported the volume of human

MT (hMT) to be 1.05cm^3, and the average volume of human MST (hMST) to be 0.38cm^3.

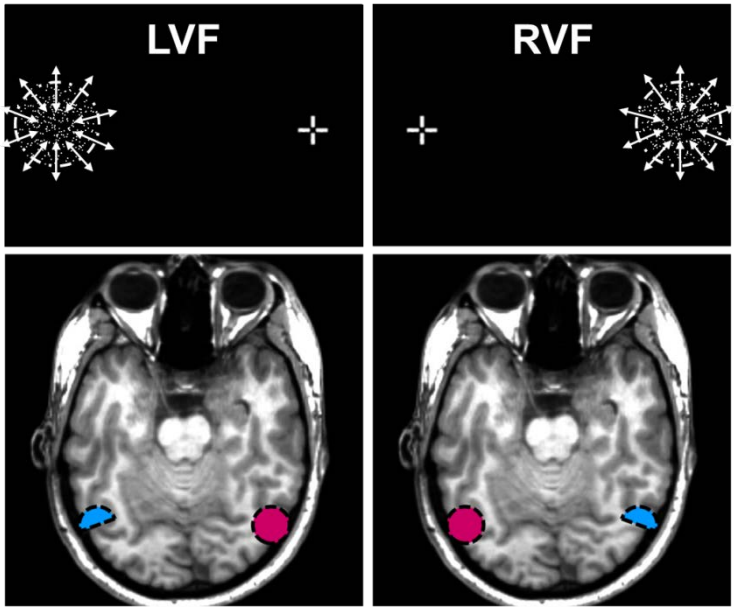

Figure 1.5. Demonstration of activation for stimuli presented in left (LVF) and right (RVF) visual fields. Pink areas represent contralateral activation of the entire hV5/MT+ complex, whereas blue semi-circles represent ipsilateral activation of the anterior portion of the hV5/MT+ region. This area correlates to area TO-2.

This experiment also posited some proposed functionality of hMST as differential responses were obtained for optic flow stimuli compared to visual and non-visual (tactile) pursuit. Dukelow et al. (2001) reported that the hypothesised anterior portion of hV5/MT+ demonstrated an increase in activation for optic flow stimuli (purported to be homologous to MSTd) whereas visual pursuit (more commonly associated with MSTl/v in the monkey) activated a large indistinguishable portion of the whole complex. It was unusual then to find that the non-visual pursuit activated a separate anterolateral portion of the hV5/MT+ complex that Dukelow et al. compared

to the monkey MSTl/v. This paper therefore concluded that there are 3 regions within hV5/MT+ including hMT, hMSTd and hMSTl/v. However, it would be expected that visual (as opposed to nonvisual) pursuit should also produce activation in putative hMSTl/v given that MSTl/v in the monkey has been frequently previously associated with eye movements (Dursteler and Wurtz, 1988; Komatsu and Wurtz, 1988; 1989). This suggests that although there are similarities between monkey MT+ and human V5/MT+, there may also be key differences.

Following this, Huk et al. (2002) managed to use a similar functional mapping technique to show that at least two areas can be reliably distinguished using this method. For this, they used a 21° aperture of white dots that moved radially, alternating every second. The nearest edge of the stimuli was displaced horizontally by 10°. This ipsilateral/contralateral distinction allowed successful identification of hMT and hMST. They also compared their results to a retinotopic map representation and found that compared to the relative over-representation of visual space in monkey MT (Maunsell and Van Essen, 1987), there was much more evidence of a regular retinotopic organisation in the human MT. There was also a clearer representation of a retinotopic map in hMT compared to anterior hMST, and this is thought to be related to increasing size of receptive fields. Neurons within this anterior region of hMT+ were found to show an increase in response to both ipsilateral and contralateral optic flow stimuli. This area in humans was compared to monkey MST as a whole, as opposed to discussing the likelihood of it being correlated with MSTd or MSTl/v. Despite this lack of proposed functionality, it was discussed that the researchers only identified two sub-divisions, as

opposed to Dukelow's potential three (Dukelow et al., 2001). This difference prompted further discussion from the authors regarding the inconsistency of these MT+ sub-divisions even across different species of monkey (Rosa et al., 1993) thereby further implying that perhaps it would be unsurprising to discover that not all MT+ sub-divisions are preserved in humans.

Since this research was published, functional imaging techniques have become advanced enough to be able to measure the retinotopic maps in these two satellites of hV5/MT+ by using model-based mapping paradigms (see Dumoulin and Wandell (2008)). Amano et al. (2009) successfully utilised this method to show that the human homologues to MT and MST appeared to exhibit clear retinotopic boundaries. The researchers labelled these areas using the anatomical nomenclature outlined previously by Wandell et al. (2007), and referred to hMT as temporal occipital-1 (TO-1), and hMST as temporal occipital-2 (TO-2) (see Figure 1.6). Further investigations allowed an analysis of population receptive fields (pRF) size of neurons within TO-1 and TO-2; showing that for both areas, pRF size increases as a function of eccentricity. This study also reinforced that the coverage of these areas encompasses a section of ipsilateral visual space, with neurons within anterior TO-2 seemingly more sensitive to ipsilateral activation than within TO-1. Amano et al. also used specialised motion localiser stimuli in order to distinguish between these sub-regions and compare the results to previous literature (Dukelow et al., 2001; Huk et al., 2002). This involved contrasting activations for contralateral and ipsilateral moving dots.

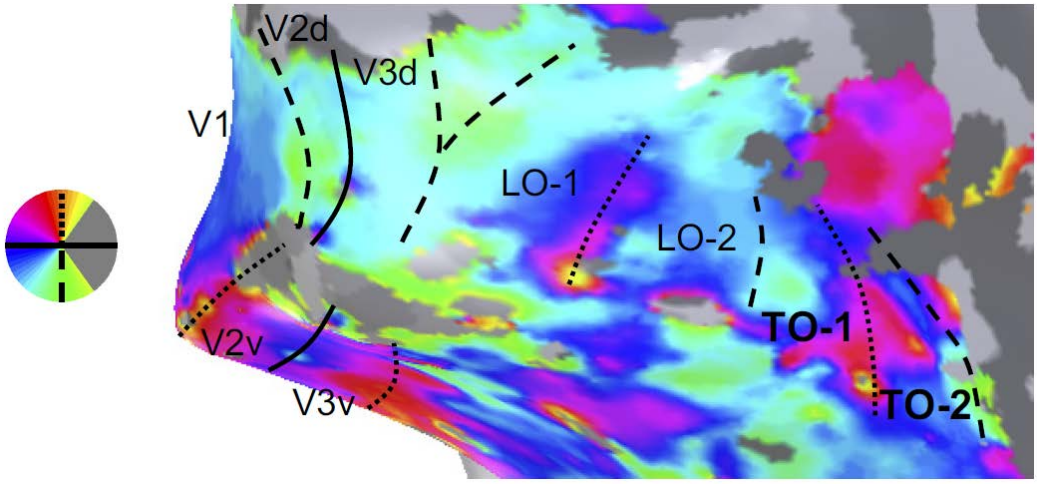

Figure 1.6. Superimposed polar angle maps from a single subject showing the full hemifield representations of TO-1 and TO-2 in the right hemisphere. Black dotted lines represent the upper vertical meridian and black dashed lines represent the lower vertical meridian. It is clear from this map that these representations are likely to be adjacent to visual area LO-2. (Adapted from Amano et al., 2009).

In terms of retinotopic organisation, Amano et al. (2009) reported that TO-1 and TO-2 share a parallel eccentricity map which increases from ventral to dorsal cortex, and it has been noted that they share an extended foveal representation. As shown in Figure 1.5, the angular representation in TO-1 extends from the lower vertical meridian at the boundary to LO-2, all the way through to the upper vertical meridian at the boundary to TO-2, encompassing a full hemifield. Similarly, the angular representation in TO-2 demonstrates a phase reversal as it extends from the upper to the lower vertical meridian.

In their discussion, Amano et al. proposed that the human visual area TO-1 most likely corresponds to monkey MT because the retinotopy of TO-1 is most similar to that of MT with posterior regions representing the lower visual field and anterior portions representing the upper visual field. This is

consistent with reports from previous studies identifying the posterior sub-division of hV5/MT+ in humans as hMT (Dukelow et al., 2001; Huk et al., 2002). However, Amano et al. also identified a slight difference between the pRF of neurons within these areas across the two species. At 5° eccentricity, the population receptive field radius estimates of TO-1 are predicted to be ~7-8° (which equates to a population receptive field width of 15°), whereas macaque MT is known to have smaller receptive fields, roughly 5-7° (Maunsell and Van Essen, 1987).

Amano et al. went on to propose that the TO-2 maps correspond most closely to the monkey visual area known as lateral MST (MSTl/v). This is based on several existing similarities between the two areas, including the correlating increase in receptive field size with eccentricity of visual field (i.e. Eifuku and Wurtz (1998)), which is not typically evident in MSTd. Also TO-2 only demonstrated a relatively slight ipsilateral representation of the visual field which is very different to the 40° ipsilateral representation reported for neurons in MSTd (Raiguel et al., 1997). However, it is also important to recognise the potential differences between TO-2 and MSTl/v. Firstly, in this experiment TO-2 was activated by radial optic flow which is found to be particularly preferential for MSTd (Saito et al., 1986; Duffy and Wurtz, 1991a). Whereas MSTl/v is functionally defined as being important for pursuit eye movements and segregating objects from their background, and is much less activated by large optic flow stimuli (Eifuku and Wurtz, 1998). Also previous research has reported that pursuit (associated with MSTl/v) activates an anterolateral area separate from an anterior portion responsive to optic flow (Dukelow et al., 2001). This highlights that it is still unclear as to

whether area TO-2 has an exact homologue in monkey visual cortex and that gaining a better understanding of the function of this area will be an important development in the next few years.

Another method for distinguishing between sub-divisions of hV5/MT+ has been described recently by Pitzalis et al. (2010; 2013b). In these experiments they were focusing primarily on investigating a medial visual motion area known as V6, but consequently they found that retinotopically mapping V6 in a human brain required the use of much wider retinotopic and functional localiser stimuli extending up to 55° into the visual periphery of each hemifield. This use of stimuli approximately 10 times larger than the standard stimuli used for retinotopic mapping allowed researchers to acquire much clearer polar angle representations within extra-striate middle temporal and parietal areas with large receptive fields. This research found multiple maps within hV5/MT+. This further supports the notion of similarity between monkey MT+ and human V5/MT+ in that this complex does appear to be made up of several smaller sub-divisions. However, it is also clear that the sub-divisions Pitzalis et al. report finding within hV5/MT+ were much greater in number than Amano et al. (2009) were able to demonstrate.

This discrepancy leads on to more recent work by Kolster et al. (2010). These researchers used a similar model-based fMRI mapping technique to propose an alternative to the TO-1/TO-2 distinctions (Amano et al., 2009). It is clear that the organisation offered by Amano et al. differs greatly from that of monkeys in both the anatomical location of the areas, and the inability to identify homologues to areas FST and V4t (cf. Kolster et al., 2009). Following this, Kolster et al. reasoned that as it is known that humans are more

sensitive to visual presentation of moving stimuli than monkeys are (Vanduffel et al., 2002), therefore it is unlikely that humans will have fewer motion areas than monkeys. Using a slight variation of the standard retinotopic technique, involving rotation periods of 8s, Kolster et al. (2010) reported they had successfully managed to differentiate between four different field representations within the hMT+ cluster that all share a foveal confluence: MT, pMSTv, pFST, pV4t (see Figure 1.7; Table 1.1).

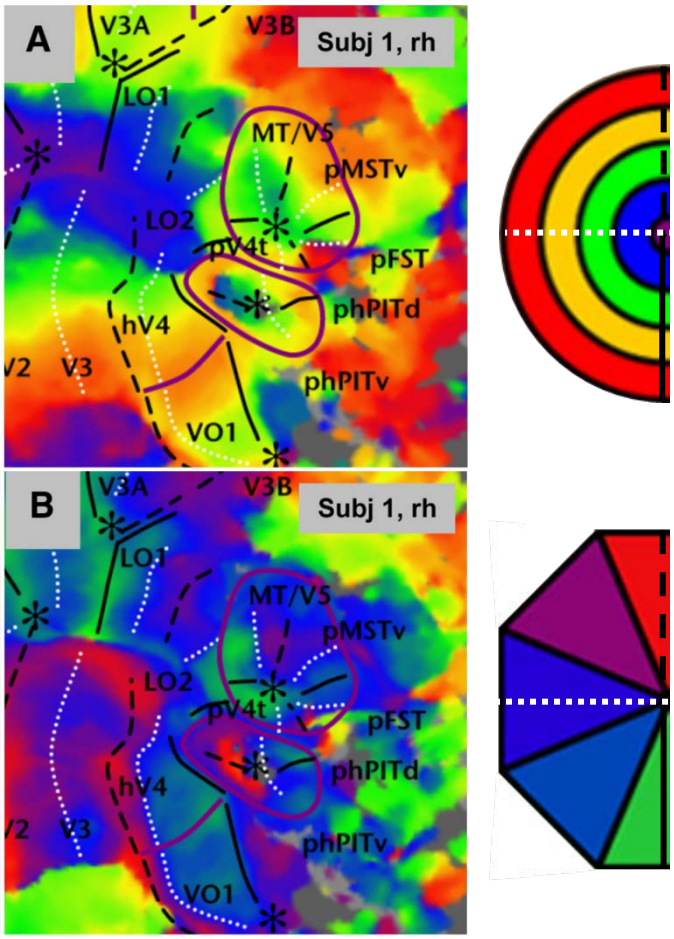

Figure 1.7. Superimposed eccentricity (A) and polar angle maps (B) on a flattened representation of an individual's cortex. The asterisks represent central visual field, with solid and dashed black lines representing lower and upper vertical meridians. The white dotted lines represent horizontal meridians and the purple lines show positions of peripheral eccentricity ridges. (Adapted from Kolster et al., 2010).

Table 1.1. Table comparing average Talairach co-ordinates for TO-1 (hMT) and TO-2 (hMST) from Dukelow et al. (2001) and Kolster et al. (2010).

	TO-1/MT			**TO-2/MST**		
Study	*x*	*y*	*z*	*x*	*y*	*z*
Dukelow et al., 2001	44 ± 3	-64 ± 7	5 ± 4	45 ± 3	-60 ± 5	5 ± 4
Kolster et al., 2010	46	-78	8	44	-70	6

Kolster et al. (2010) examined the retinotopic and functional properties of these areas and managed to show that MT is very sensitive to motion in general, pMSTv is greatly activated by random dot patterns, and that pFST and pV4t have stronger shape selectivity. This is in accordance with monkey literature suggesting that MST is more activated by complex dot patterns than MT, and that FST and V4t are involved in ventral form processing (Tanaka and Saito, 1989; Duffy and Wurtz, 1991a; Duffy and Wurtz, 1991b; Nelissen et al., 2006).

Kolster et al. (2010) also discuss a few possible explanations as to why previous studies may not have been able to locate four motion maps in hV5/MT+. Firstly they proposed that the 90° wedge stimulus used by Huk et al. (2002) was potentially too wide to be able to accurately represent the larger receptive field sizes in these extra-striate areas. Secondly, the short 3s rotation period of wedges during the mapping Amano et al. (2009) may have produced difficulty in consistently identifying the lower quadrant of TO-2. However, regardless of methodology, these two new proposed subdivisions of hV5/MT+ are only very small regions of cortex, with pFST encompassing ~ 132mm^2, and pV4t only encompassing ~106.5mm^2. This means that if they are functionally dissociable from areas MT (TO-1) and MST (TO-2), then it will be very difficult determine using fMRI or TMS because the spatial

resolution of these techniques is too limited at the present time. This means that research will probably remain focused on the main two areas (TO-1 and TO-2) until the technology is able to catch up with these developments.

Previous literature consistently reports that there are at least two maps in hV5/MT+ (Huk et al., 2002; Amano et al., 2009; Kolster et al., 2010), and it is thought that if there exists more than one map then it can be assumed they are most likely performing separate functions. This therefore means that the next logical step is to discover the functional properties of these two motion areas and to attempt to better clarify the differences between motion areas separated from hV5/MT+ (i.e. V3A).

One proposed functional difference, in accordance with the differences reported between monkey MT and MST, is that the anterior region (TO-2) is more specialised for encoding global flow properties. Smith et al. (2006) have shown that this anterior motion-sensitive region in humans (which they refer to as MST) shows a greater percentage of signal increase for all types of motion (expansion, complex, rotation, translation, random) compared to MT when contrasted with blank fields. Similarly, MT shows preferential activation for coherent motion compared to random motion and this effect is more evident for complex (spiral) motion than any other kind. They conclude that the anterior region is much more involved in these global flow types of motion than the posterior region. Similarly, Wall et al. (2008) reportedly found that neurons within human MST became adapted to expanding and rotating stimuli, suggesting that they have a preference for this type of motion. Following this, as fMRI data can only assert a correlational relationship between stimuli and cortical regions, a way to establish causal relationships

would be advantageous. This would be possible using a method such as transcranial magnetic stimulation (TMS) to disrupt each area individually whilst the subject performed different motion-related tasks. Such a technique would enable the ascription of specific functional roles to these motion areas. This is the approach adopted in the research described below (i.e. identify sub-divisions using fMRI paradigms, and use TMS to selectively disrupt them; fMRI-guided TMS).

1.3.4 Motion Sensitive Area V3A

As previously discussed area hV5/MT+ is not the only motion-sensitive area within the human brain. One area of particular interest to this thesis is V3A which is located adjacent to V3 (dorsal) near the occipital pole (see Figure 1.1).

Functional neuroimaging data has shown that this area demonstrates an increase in BOLD signal for moving stimuli; particularly first-order motion (Tootell et al., 1997; Smith et al., 1998), whilst TMS experiments have shown that this area is contained within a network of regions that are responsible for processing speed of both luminance and chromatically-defined motion (McKeefry et al., 2008; McKeefry et al., 2010).

A further proposed function of V3A is the identification of the centre of expanding/contracting (radial) stimuli, referred to as the focus of expansion (FOE). Neuroimaging experiments have established that the position of this FOE correlates with the location of activity within the retinotopic representation contained within V3A (Koyama et al., 2005). This implies that

V3A is able to process the location of the FOE without necessarily contributing to the processing of the radial motion. Support for this is found in a case study from Beardsley and Vaina (2005a). These researchers found that a patient (GZ) with damage to right hV5/MT+ had reduced ability to discriminate radial motion (expanding/contracting), whilst maintaining ability to identify clockwise/anti-clockwise rotational motion and an ability to identify the FOE of radial motion. This data seems to suggest that another area may be responsible for the localisation of the FOE, which in GZ's case may potentially be the preserved area V3A. However, case studies are based on an individual with gross-scale damage to the cortex and it can therefore be quite difficult to extrapolate these findings to the normal population. The most appropriate way to determine the definitive role of V3A in the processing of FOE will be to design a transcranial magnetic stimulation experiment in order to determine causality.

1.3.5 Other Motion Sensitive Areas

Other less studied motion-sensitive areas include the intra-parietal sulcus (IPS) and medial area V6 (Braddick et al., 2000; Pitzalis et al., 2010; Pitzalis et al., 2013c); as indicated in Figure 1.8. The IPS in the human brain is thought to contain up to five sub-divisions extending from IPS-0 to IPS-4 (Swisher et al., 2007; Wandell et al., 2007), though it should be noted that IPS-0 is also sometimes referred to as V7 (Tootell et al., 1998), and IPS-1/2 are sometimes referred to as medial posterior parietal cortex, or mPPC (Bartels et al., 2008). This section of the visual cortex is often associated with

perception of coherent motion stimuli, which likens it to hV5/MT+ (Braddick et al., 2000; Pitzalis et al., 2013c).

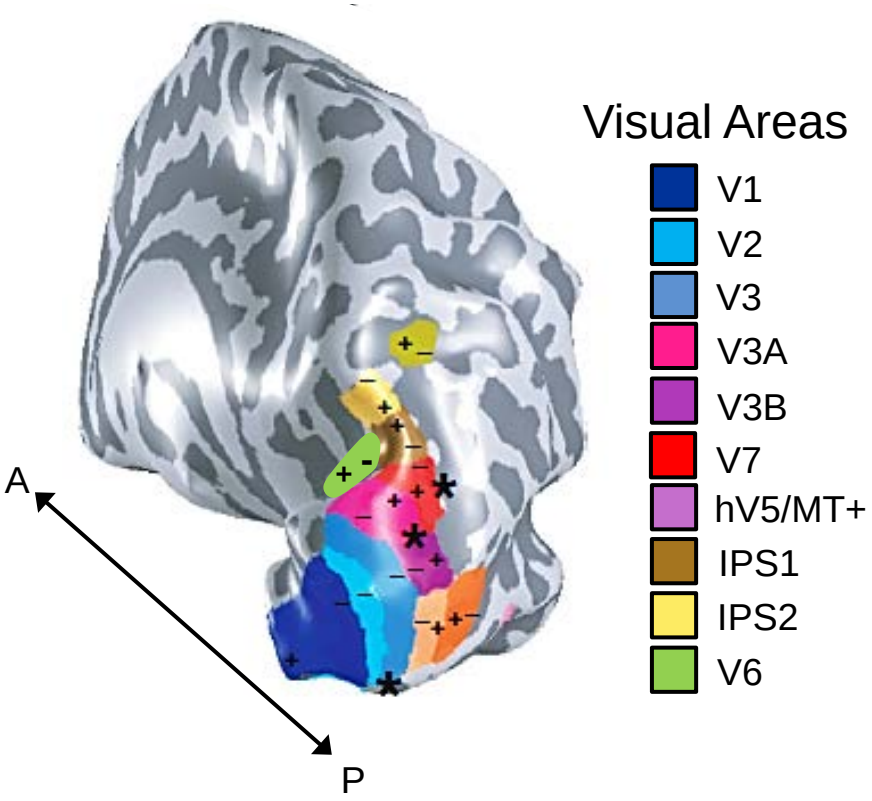

Figure 1.8. Adapted figure showing location of visual areas on inflated right hemisphere (A = anterior; P = posterior). Dark grey patches indicate sulci, whilst light grey represents gyri. Superior visual field representations (+), inferior visual field representations (-), and foveal confluences (*) are shown for each area. (Adapted from Wandell et al., 2009).

These posterior parietal regions (particularly IPS-1/2) have been shown to be responsive to optic flow (Bartels et al., 2008), and positron emission tomography (PET) evidence has demonstrated that the dorsal IPS region is one of many that contribute to a network of visual areas that determine heading or self-motion (Peuskens et al., 2001). Magnetoencephalography (MEG) experiments have also indicated an important role of medial parieto-occipital areas in the processing of self-motion (Tikhonov et al., 2004). Taken

together, this literature proposes a similarity between human IPS and non-human primate ventral intraparietal area (VIP) as it has been shown that this area encodes heading information in macaque monkeys (Bremmer et al., 2002).

There is also evidence of these parietal regions being involved in higher-order cognitive processes such as attention. Neuroimaging experiments have highlighted that attentive tracking (as opposed to passive viewing) of moving objects incurs increases in activation in the IPS region (Culham et al., 1998). This suggests that the IPS region may process motion that is being actively attended to, and also proposes that these areas may have links to regions responsible for processing eye movements.

Another motion-sensitive area near the IPS region is visual area V6. This area is usually located medially in the parieto-occipital sulcus and is often associated with processing of coherent motion, flow field patterns, and ego/self-motion (Pitzalis et al., 2010; Cardin and Smith, 2010; Cardin and Smith, 2011). However, despite this area producing activation for expanding flow-field stimuli (Pitzalis et al., 2010; Pitzalis et al., 2013a), it has been shown that neurons in V6 do not seem to process the position of the central focus of the expansion (FOE) (Cardin et al., 2012). Instead, this area appears to be involved in the overall analysis of self-motion, particularly in the integration of visual depth and flow cues (Cardin and Smith, 2011). Visual area V6 in non-human primates is also known to receive input from dorsal motion-sensitive area MST, which further suggests a role in the integration of visual flow signals (Galletti et al., 2001).

Recent investigations of speed of signal transfer using visually evoked potentials (VEPs) has shown that area V6 in humans produces a VEP that is almost simultaneous with that of area hV5/MT+ (Pitzalis et al., 2013a). If this is considered further, it seems likely that V6 and hV5/MT+ must be responsible for processing different aspects of visual motion, as it implies the pathway from the occipital pole to each respective area is processed in a parallel, not serial manner. These researchers also reported a late second VEP from V6 which they interpreted as being feedback from an extra-striate motion area such as V3A (Pitzalis et al., 2013a). In this respect, it seems that this area may be responsible for combining signals from other lower areas in order to contribute to the final overall percept of self-motion.

1.4 Transcranial Magnetic Stimulation

Transcranial magnetic stimulation (TMS) is a technique that can be used to "induce a transient interruption of normal brain activity" (Walsh and Cowey, 2000, p.74). It bases its fundamental principles on Faraday's law of induction (Wagner et al., 2009); essentially utilising the principle that an electrical current passed through one coil creates a magnetic field which can then induce a current in an adjacent coil. With TMS, the electrical current is passed through the TMS coil and the subsequent magnetic field induces electrical potentials in localised regions of human cerebral cortex (O'Shea and Walsh, 2007). It is thought that this creates 'neural noise' as it increases the signal-to-noise ratio in these regions of cortex (Walsh and Cowey, 2000). This presents itself as impaired performance on a range of tasks, including

tasks involving motion and speed perception (Walsh et al., 1998; Pascual-Leone, 2001; McKeefry et al., 2008; Thakral and Slotnick, 2011).

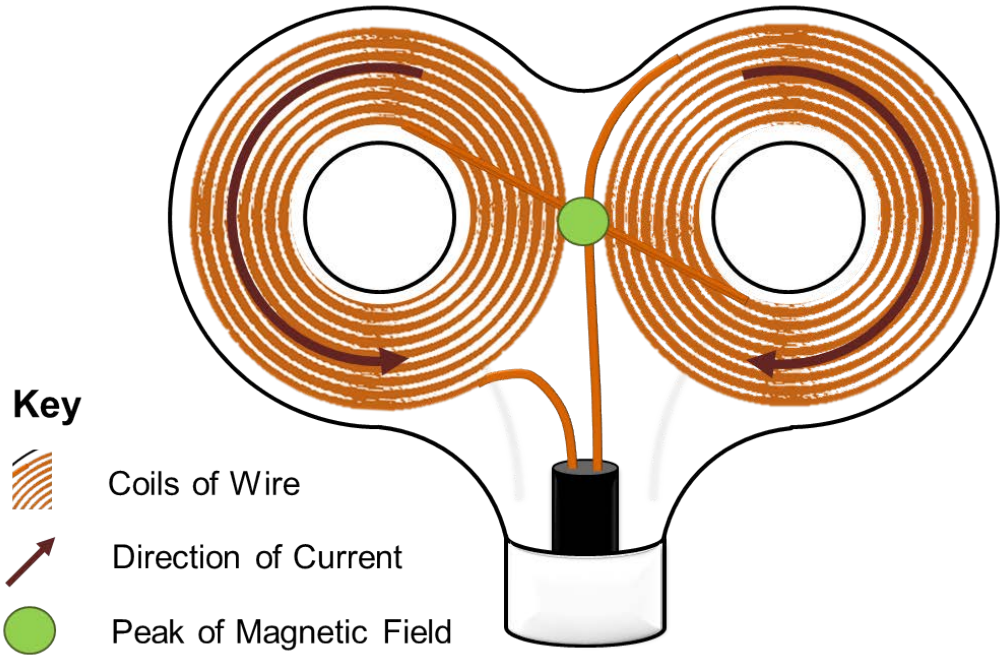

Figure 1.9. Simple diagram outlining the internal make-up of a Magstim figure-of-eight coil. The green circle shows the positioning of the magnetic pulse, the brown arrow shows the direction of current within the coil and the orange coils represent the coils of wire.

A key advantage of this technique is that the magnetic pulse initiated by the coil is able to move freely through skin, muscle and bone without losing any strength or intensity. This means it is able to stimulate the cortical neurons without the need for invasive procedures (Hovey et al., 2003). A typical 50mm figure-of-eight coil (Figure 1.9; 1.10) will produce a magnetic pulse that will affect parallel cortical neurons directly underneath the centre of the coil (Roth and Basser, 1990; Rossi et al., 2009). This pulse can travel to a depth and breadth of approximately 1-2cm, making it a useful coil for very

localised stimulation (Thielscher and Kammer, 2004; Zangen et al., 2005; Roth et al., 2007).

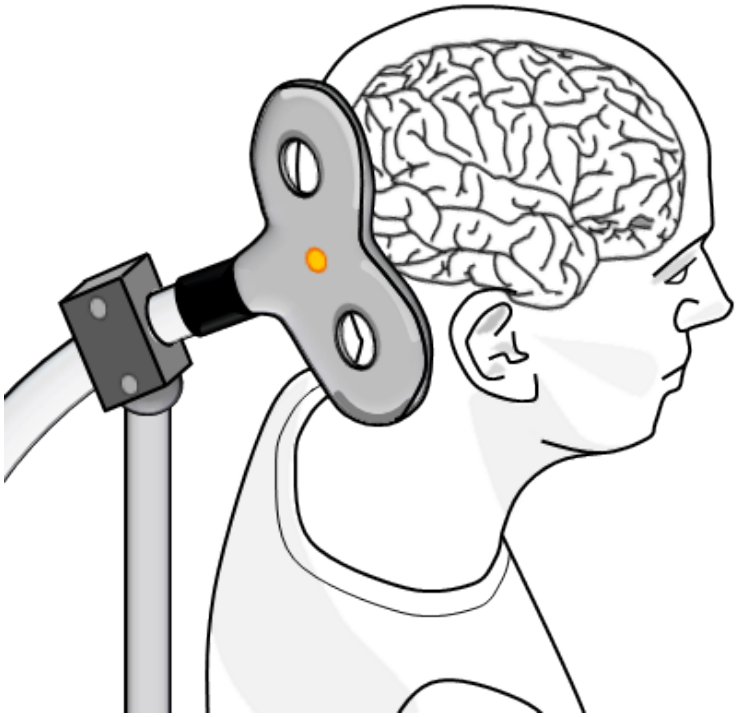

Figure 1.10. Diagram outlining the application of TMS to a subject's head. The orange circle outlines the peak positioning of the magnetic pulse.

The biggest advantage of using TMS to functionally dissect the visual cortex is that it provides casual evidence for functionality. Relying solely on data obtained from fMRI or other neuroimaging methods can only assert that a brain region is involved in a particular task (correlative), whereas TMS can assert that the brain region is necessary for the task (causal).

However, despite the potential benefits of using TMS, there can be discrepancies in the accuracy of targeting and localising the regions of interest in each subject. Some researchers measure from anatomical external locations on the outside of the head i.e. hV5/MT+ is thought to be in an area that correlates with the part of the head found 3cm dorsally and 5cm

laterally from the inion (Campana et al., 2002). Another way of identifying this motion-sensitive region was to utilise the known effects found when successful application is achieved. For example, it is known that TMS to hV5/MT+ can induce moving phosphenes (an afferent spot of visual light produced without light actually entering the eye). Following this, it can be asserted successful identification of moving phosphenes would correlate with successful identification of hV5/MT+ (Silvanto et al., 2005). However, it is not always possible to produce moving phosphenes in every participant (Pascual-Leone and Walsh, 2001), and it is also reportedly possible to create moving phosphenes by stimulating regions of cortex that neighbour hV5/MT+ (Fernandez et al., 2002). However, recent evidence of the large-scale individual variability in the anatomical location of extra-striate visual areas has demonstrated how unreliable it is to use external anatomical landmarks to accurately localise stimulation sites (McKeefry et al., 2009). It is even unreliable to use cerebral anatomical landmarks as there is likely to be great variability in the structure of the gyri and sulci in the area of cortex surrounding hV5/MT+. For example, hV5/MT+ is reported to be located in the ascending limb of the inferior temporal sulcus (Dumoulin et al., 2000), however this is an average location and will vary between individual subjects. The reason accurate localization is such an important factor is because the motion sensitive areas (TO-1 and TO-2) are very small; ~258-265mm^2 (Amano et al., 2009). This means that even a slight shift in the position of the coil may be sufficient to abolish the effects of TMS. It is therefore clear that it will be extremely important to be as accurate as possible when localising these areas in subjects.

Following this assertion, it is widely held that the most reliable method of localising TMS pulses is to use fMRI-guided TMS (Sack et al., 2006; McKeefry et al., 2009). This is a combination of techniques in which an fMRI scan (functional or retinotopic) is performed in order to identify the regions of interest in each individual subject. These regions of interest can be saved as 'target points' in 3D space, which is computed in a 3D mesh of the structural MRI scans. This mesh can then be calibrated with the position of the subjects' head along with the position of the TMS coil which allows accurate initial positioning of the coil and real-time monitoring of the application of pulses.

Once the TMS is set up, the delivery of the pulses can begin. However, there are several different ways in which to deliver the TMS (single pulse, repetitive, distal) and each of them produce slightly different effects (Walsh and Cowey, 2000; O'Shea and Walsh, 2007). Single pulse TMS is a common way of testing the effects of cortical disruption in which a single pulse is applied at each onset, whereas if a train of several biphasic pulses is applied at each onset, this is called repetitive TMS or rTMS. This repetitive technique has frequently been used to test behavioural performance on several motion perception tasks (McKeefry et al., 2008; Burton et al., 2009; McKeefry et al., 2010). These types of stimulation are usually concurrent with the task, i.e. subjects experience TMS and the stimuli at the same time.

Whereas a slightly less common technique involving delivering very low frequency (<1Hz) TMS prior to the behavioural testing is called 'offline' or 'distal' TMS (Walsh and Cowey, 2000; Thakral and Slotnick, 2011). These low frequency pulses are purported to reduce cortical blood flow to the region

being stimulated; thereby producing a short-term attenuation of behavioural ability in subsequent tests (Walsh and Cowey, 2000). For this method, the subject has TMS pulses delivered to the region of interest for a fixed period before the experiment, and then will usually not have any TMS delivered during the actual testing. This is thought to produce effects that will last between 50-200% of the TMS duration period (Walsh and Cowey, 2000; Matsuyoshi et al., 2007). For example, a 10 minute period of TMS delivery should produce effects that will last roughly between 5-20 minutes.

One important consideration when applying TMS is the moment in which to begin the onset of stimulation in order to obtain the most measurable effect. Using hV5/MT+ as an example, some studies have reported that the most effective time to deliver TMS is 60-80ms post stimulus offset (Silvanto et al., 2005), whilst others have reported success 100-150ms post stimulus onset (Hotson et al., 1994; Anand et al., 1998; Sack et al., 2006). In contrast to this, other researchers suggest it takes 30-40ms for the signal to reach V5 and therefore TMS would need to be applied before the 20ms post-onset period (Beckers and Zeki, 1995). This would include any time between -20ms and +10ms with the most effective onset time being synchronous with stimulus onset (0ms).

It is clear from these discrepancies that the temporal properties of TMS are still under consideration, and therefore for any experimental paradigm it would be prudent to use the most consistently reliable method which is to apply TMS synchronous with stimulus onset (McKeefry et al., 2008; McKeefry et al., 2010; Silson et al., 2013).

Overall, delivering fMRI-guided TMS to the different motion-sensitive sub-divisions within the human visual cortex should be a reliable way of determining the dissociable functions of these areas. It will also be interesting to compare these results to pre-existing knowledge regarding the monkey visual cortex in order to discover whether these areas are homologous or heterologous.

1.5 Research Aims and Objectives

The overall aim of this research was to functionally distinguish between motion-sensitive areas in the human visual cortex, using fMRI-guided TMS in order to determine causality. Our primary aim was to functionally segregate area hV5/MT+ into two previously identified smaller areas; TO-1 and TO-2. Previous human neuroimaging research has revealed potential functional differences between TO-1 and TO-2 for processing radial and rotational motion, however, as this evidence implies a correlative relationship between tasks and neuronal activity, it would benefit from the application of TMS in order to identify a causal link.

The secondary aim of this research was to investigate the functionality of area V3A, in particular focusing on any functional differences that might exist between the hV5/MT+ regions and V3A in radial motion tasks.

This research intends to use fMRI to identify the motion-sensitive regions of interest (ROIs), and then use two different types of TMS (‘distal’, and repetitive TMS) to investigate the functions of TO-1, TO-2, and V3A with

respect to motion coherence for two main psychophysical tasks: direction discrimination (translational, radial, rotational), and focus of expansion (FOE) localisation (radial).

Chapter 2
Methodology and Visual Stimuli

2.1 Introduction

The experiments described in this thesis used a combination of neuroimaging, neurostimulation and psychophysical techniques in order to investigate the functions of various motion areas within human cortex. This was achieved by designing psychophysical experiments characterised by different aspects of motion processing (direction, motion type, and position) and applying TMS to known motion-selective areas in order to measure the potential disruption on performance. Although the particular stimuli varied between experiments, the localisation of the target sites for TMS and the fMRI parameters were consistent throughout. For all experiments within this thesis, a total of four target sites were required to be localised in each individual subject: TO-1, TO-2, V3A and LO-1. These sites were identified using functional localisers and retinotopic mapping paradigms. Cortical area LO-1 is not motion-selective but is located in close proximity to both hV5/MT+ and V3A which makes it an optimal choice for a control site. These fMRI-guided target sites were important for ensuring application of TMS was accurate and consistent between subjects and across tasks. Any performance deficit measured following application of TMS to one of these areas would indicate that the area is necessary for the task; therefore it was also important to ensure that the psychophysical tasks were designed appropriately.

2.2 Structural and Functional fMRI Parameters

All functional magnetic resonance imaging data were acquired at York Neuroimaging Centre (YNiC, University of York). All experiments were approved by YNiC Ethics Committee. Both structural and functional scans were undertaken using a GE 3-Tesla Sigma Excite HDX scanner. The multi-average, whole-head T1-weighted structural scans for each participant encompassed 176 sagittal slices (TR=7.8ms, TE=3ms, TI=450ms, FOV=290 x 290 x 176, 256 x 256 x 176 matrix, flip angle=20°, 1.13 x 1.13 x 1.0mm^3). For the best field homogeneity of the structural data, an eight-channel 'birdcage' phased-array coil was used. These structural scans were necessary for accurate co-registration during the TMS phase of the experiment.

For functional scans, the eight-channel coil was replaced with a 16-channel phased-array half-head coil. In contrast to the evenly spaced eight-channel phased-array coil, the 16-channel coil contains 16 coils densely positioned underneath the subjects head near the occipital pole. This provided much greater sensitivity to MR signals within the posterior visual cortex, whilst signals from irrelevant anterior portions of the head were poorer.

Voxel size for the functional localisers was chosen to be 1.5 x 1.5 x 1.5 mm^3 as this allowed more accurate measurement of blood oxygenation-level dependent (BOLD) signals within smaller visual areas such as hV5/MT+. This also restricted the distortion that could potentially arise from examining a large voxel that may straddle the bank of a sulcus. This is particularly important for area hV5/MT+ as it is likely to reside within the ascending limb

of the inferior temporal sulcus (Dumoulin et al., 2000). However, this small voxel size, combined with the technical limitations of the fMRI scanner, meant the overall coverage/prescription was greatly reduced to 39 axial slices encompassing only 58.5mm (see Figure 2.1). This meant it was necessary for our prescriptions to be as accurate as possible and this was achieved by using an initial localiser scan to locate the parieto-occipital sulcus and the ascending limb of the inferior temporal sulcus (Dumoulin et al., 2000). Overall if the prescription covered both these regions then it was likely to also cover motion-sensitive areas V3A (inferior parieto-occipital sulcus) and hV5/MT+ (ascending limb of inferior temporal sulcus).

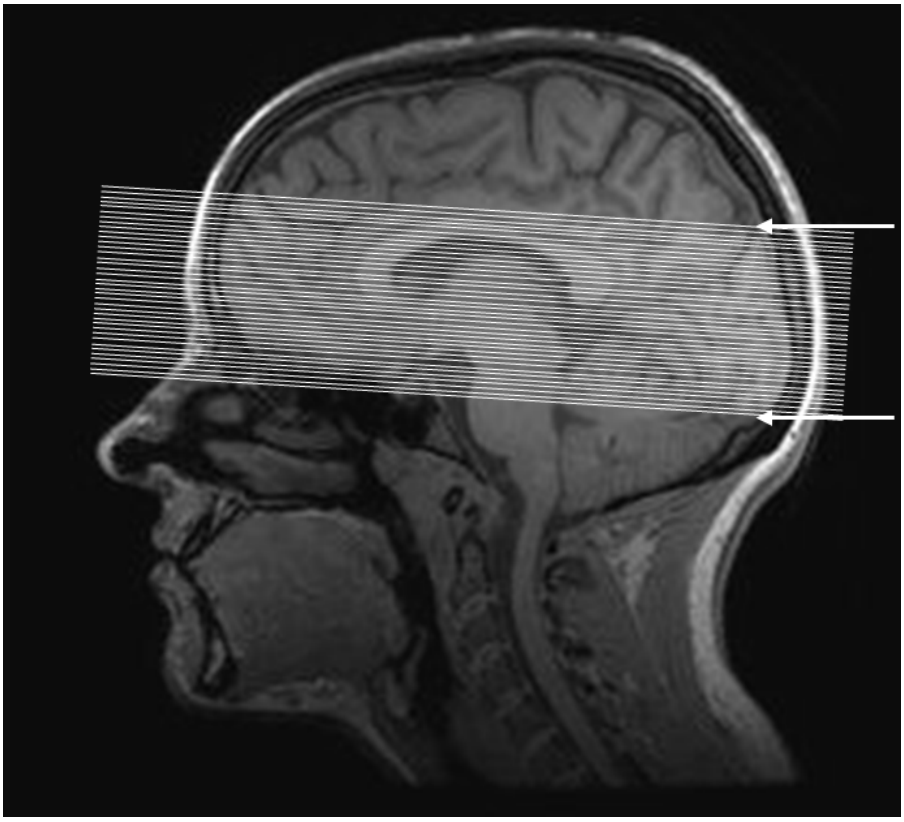

Figure 2.1. An example of the prescription of 39 slices, usually angled and positioned to provide optimum coverage of the motion areas V3A (located below the parieto-occipital sulcus (top arrow)) and hV5/MT+ which involved the bottom slice being positioned at the lowest point of the occipital lobe (bottom arrow).

The functional MRI scan used gradient recalled echo pulse sequences to measure proton density weighted images (repetition time (TR)=3000ms, echo time (TE)=29ms, FOV=192mm, 128x128 matrix, 39 contiguous slices, 1.5 x 1.5 x 1.5mm^3, interleaved slice order with no gap). Every 3s, the scanner imaged 39 1.5mm-thick slices of prescribed cortex for a duration of 300s (5 runs of a 60s block). These images provide a measurement of blood oxygenation level-dependent (BOLD) signal as a function of time. Images were read out using an EPI echo planar imaging sequence. Magnetisation was allowed to reach a steady state by discarding the first three volumes; an automated feature of the GE scanner.

2.3 Functional and Retinotopic Identification of Motion-Sensitive Target Areas

Visual stimuli were presented to participants using Psychophysics Toolbox Version 3 (Brainard, 1997; Pelli, 1997; Kleiner et al., 2007) in 32-Bit MATLAB (Version 7.6.0 Natick, Massachusetts: The MathWorks Inc., 2008). These stimuli were viewed in the MRI scanner by means of projecting images onto a custom in-bore acrylic rear projection screen (subtending 45°x30° visual angle) using a Dukane 8942 ImagePro (4500 lumens) LCD projector. The subject viewed this screen reflected in a mirror with the approximate distance from eye to screen of 57cm (Figure 2.2).

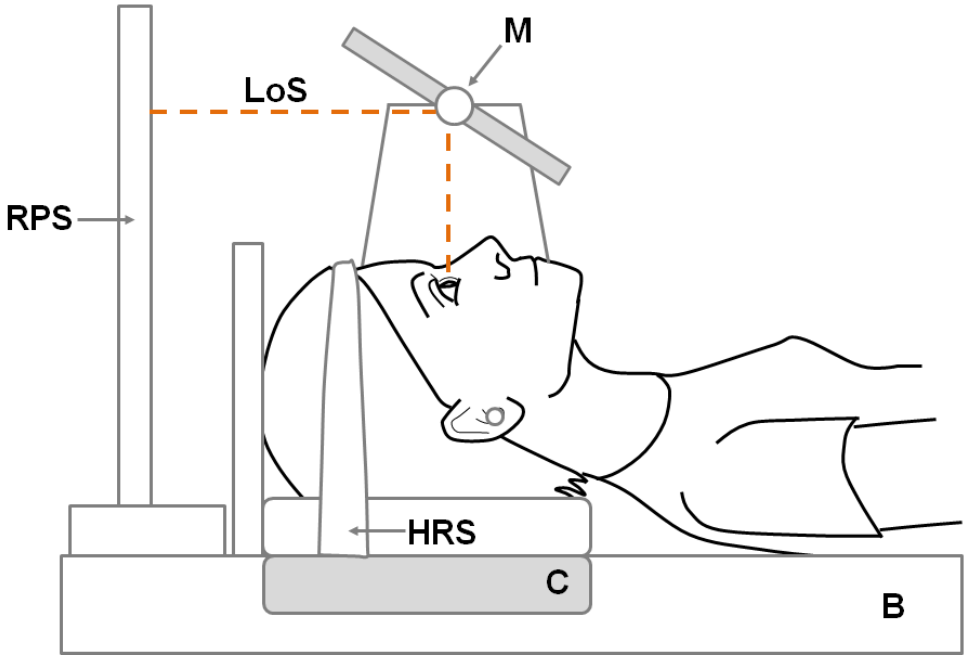

Figure 2.2. Schematic of how subject viewed projected screen with 16 channel surface coil (C). Mirror (M) connected to MRI bed (B) reflected anything projected onto rear projection screen (RPS) down the line of sight (LoS). Subject was also required to wear a head-restraint strap (HRS) to prevent movement during scanning.

2.3.1 Functional Localisers

In order to accurately functionally localise target areas TO-1 and TO-2, it was necessary to utilise a technique that has previously been successful in other laboratories (Dukelow et al., 2001; Huk et al., 2002; Amano et al., 2009). This particular technique involved presenting moving dots in both the left and right peripheral visual fields (in separate sessions). This peripheral stimulation is known to activate the majority of the contralateral hV5/MT+ complex, whilst restricting any ipsilateral activation to just the anterior portion of hV5/MT+. This anterior portion is thought to correspond to anterior area TO-2. Consequently, presenting these peripheral dots to both the left and right visual field (independently) should produce ipsilateral activation in both

the left and right TO-2. It is then possible to subtract this division from activation within the whole complex to estimate the location of TO-1.

The specific parameters of the stimuli were designed to correlate with those used previously (Huk et al., 2002; Amano et al., 2009). One localiser horizontally displaced moving dots 10° into the periphery of the left visual field, whilst the other localiser horizontally displaced the dots 10° into the periphery of the right visual field (see Figure 2.3). Each cycle began with 15s of a blank screen and a fixation cross, followed by 15s of 'moving' stimuli, 15s blank and 15s 'static' stimuli (see Figure 2.4). The 'moving' condition comprised a 15° circular aperture containing 300 dots (~0.2°) moving 8°/s radially inwards and outwards with the direction alternating every 1 second. The 'static' dots were positioned in exactly the same circular aperture as the 'moving' dots, but they remained stationary throughout the block. Comparing BOLD activation within the brain for 'moving' and 'static' conditions allowed us to confidently assert that any activation observed was a result of that particular brain region preferring moving dots over stationary dots. The blank periods between stimulus presentations served as buffers to let haemodynamic responses within cortical regions of interest return to baseline levels. This was important for distinguishing between conditions as it ensured that activation seen in one condition was due to the presented stimulus and not simply an artefact of temporal adjacency to a separate condition.

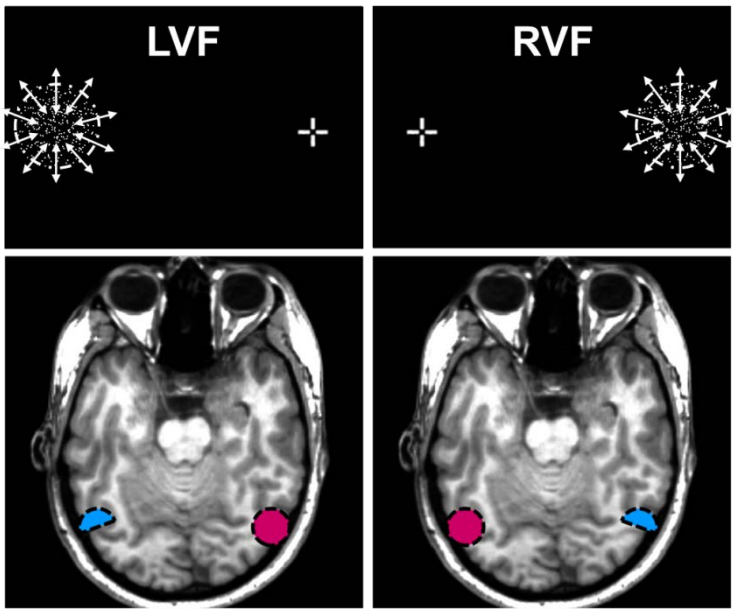

Figure 2.3. Approximate expected pattern of activation for each of the TO-2 localisers. Pink areas represent contralateral activation of the entire hV5/MT+ complex, as is usual when viewing moving dots, whereas blue semi-circles represent ipsilateral activation of the anterior portion of the hV5/MT+ region. This area corresponds with area TO-2.

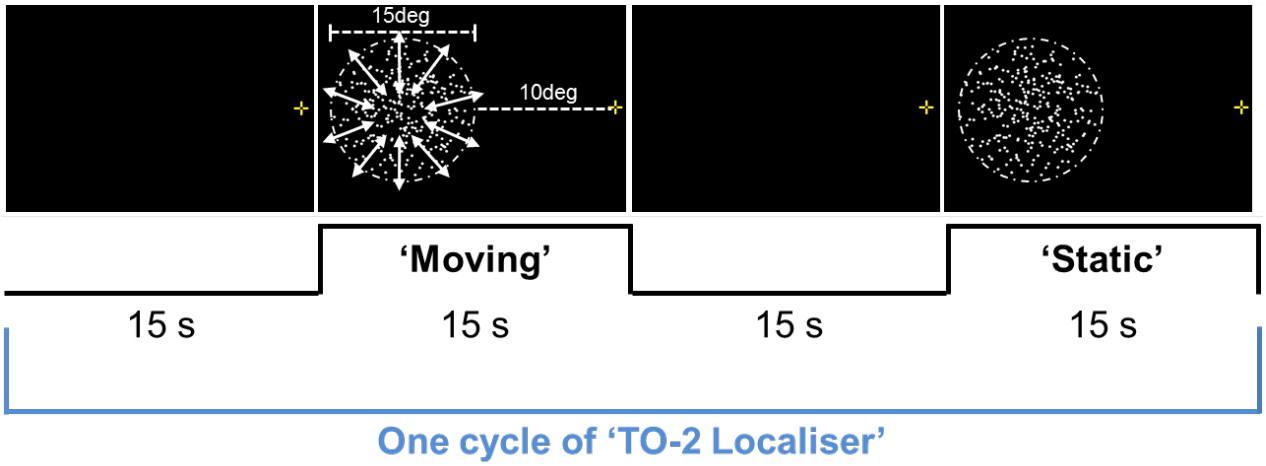

Figure 2.4. Time course of example 'TO-2' localiser presenting dot aperture in left visual field. Schematic also shows dimensions of apertures and direction of dots.

2.3.2 Retinotopic Mapping Stimuli

In order to map cortical representations of the visual field, a process known as retinotopic or 'phase-encoded' mapping was used. This involved

presenting subjects with 100%-contrast flickering checkerboard stimuli in the shape of either a ring or a wedge (Engel et al., 1997). Stimuli were generated with MATLAB and controlled by MatVis (Neurometrics Institute, Oakland, CA). The ring-shaped stimuli each comprised of 3 rings of the checkerboard and progressed from fixation to periphery as increasingly eccentric presentations in 8 transitions (to a maximum of 15° radius). As the largest and final ring reached the eccentric limits of the visual field, a small ring was positioned back at the centre of the screen to start the run again. Similarly, the wedge stimulus comprised a 90° wedge of the checkerboard and rotated anti-clockwise about the centre of the screen (point of fixation) in 24 steps. For both types of stimuli, the background was mean-grey, and the contrast reversal rate (flicker) was 6Hz (see Figure 2.5). The complete time course of one cycle for both types of stimuli was 36s, and one complete run would consist of 8 full cycles. For these experiments, the ring stimuli were only presented for one run, whereas the wedge stimuli were shown for a minimum of 3 runs and the phase-encoded data for each run were averaged together in the analysis.

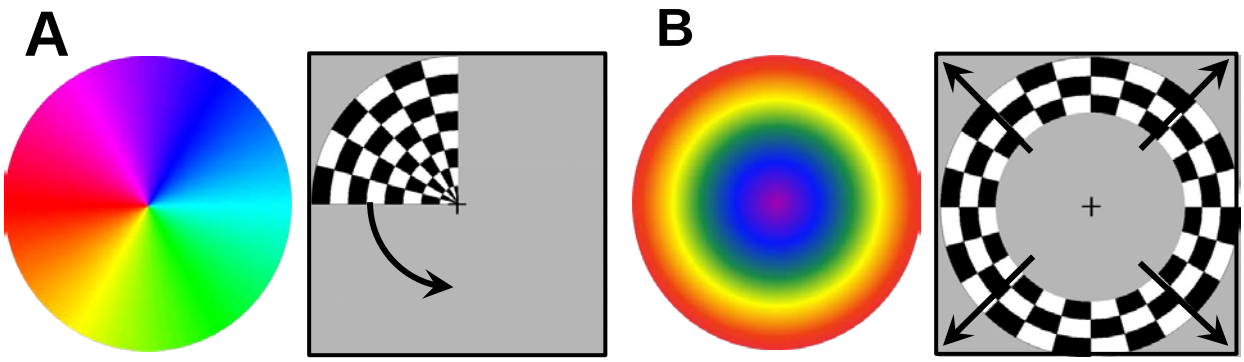

Figure 2.5. Example wedge (A) and ring (B) stimuli with corresponding colour phase maps.

2.4 Preprocessing

Preprocessing of fMRI data was a necessary step in order to ensure that the data was analysed appropriately and any conclusions were extrapolated accurately. During a single scanning session, the subject was instructed not to move and their head was restrained either with a strap (16 channel coil) or restrained by cushioning placed on either side of the head (8 channel coil). Despite this however, natural residual movements still occur as an artefact of breathing and having blood pumped around the body. The appropriate solution to this involved spatially smoothed the data by convolving the fMRI signal with a Gaussian function. In these experiments the size of the Gaussian kernel was measured as a Full Width at Half Maximum (FWHM) of 3mm.

Another stage of preprocessing involved a process of 3D motion correction to account and correct for any movements that may have occurred during the scan that would then affect the reliability of the data. The 3D motion correction used the first volume as a reference and then subsequently sorted through the volumes in ascending order using a trilinear/sinc interpolation.

Using Brainvoyager QX, it was also possible to run slice scan timing correction algorithms which use cubic spline interpolation to correct for the assumption that all slices taken within a volume were acquired at the same time. A final preprocessing algorithm that worked to improve statistical power of data analyses was high-pass (GLM-Fourier) temporal filtering (0.01 Hz). This filter corrected any low-frequency drifts in time-courses of voxels. This worked by allowing high-frequency stimulus-related activity to pass and be

included in the final analyses, whilst removing low-frequency noise-related activity. The final step of the preprocessing stage involved manually aligning the functional axial prescribed scans with the full-head structural scans in order to be able to appropriately overlay the data onto the 3D structural mesh.

Once every individual data set had been pre-processed then data from the TO-2 localiser scans were averaged together and analysed using a multi-study general linear model (GLM) in BrainVoyager QX (Brain Innovation, Maastricht). This GLM compared BOLD activation for all ‘moving’ and ‘static’ conditions for the TO-2 localiser in each visual field. This allowed comparisons to be made between regions of interest that showed increases in activation for contralateral and ipsilateral moving stimuli.

Retinotopic mapping data was analysed by computing an analysis of the travelling wave using correlation analysis in the Stanford mrVista toolbox (Teo et al., 1997; Wandell et al., 2000). This analysis stream corrected alignment and timing but did not smooth the data. It also computed the coherence, phase and signal amplitude of the response to the travelling wave at all voxel locations in the dataset. This phase map was then assigned a colour-wheel of pseudo-colour which was superimposed onto the structural scan for visualisation of the pattern of response.

2.5 fMRI Results

2.5.1 Identification of TO-1 and TO-2

Using the standard method of applying general linear models (GLMs) contrasting ‘moving’ with ‘static’ protocols for data acquired from the TO-2 localiser scan sessions, ipsilateral BOLD responses within hV5/MT+ were identified in 13/14 hemispheres tested. Of these 13 hemispheres, stimuli presented in the left visual field produced a significant cluster of activation in anterior left hV5/MT+ (left TO-2), and stimuli in the right visual field produced activation in right anterior hV5/MT+ (right TO-2) which is consistent with our hypothesis regarding ipsilateral RF coverage of TO-2 (Amano et al., 2009). This ipsilateral activation of anterior TO-2 was only evident in subjects that viewed at least 3 runs of the localiser (total 15 ‘moving’ and ‘static’ conditions). Posterior TO-1 was then identified by subtracting the anterior TO-2 activity from the whole hV5/MT+ complex activation found for contralateral presentations (Figure 2.6; Figure 2.7).

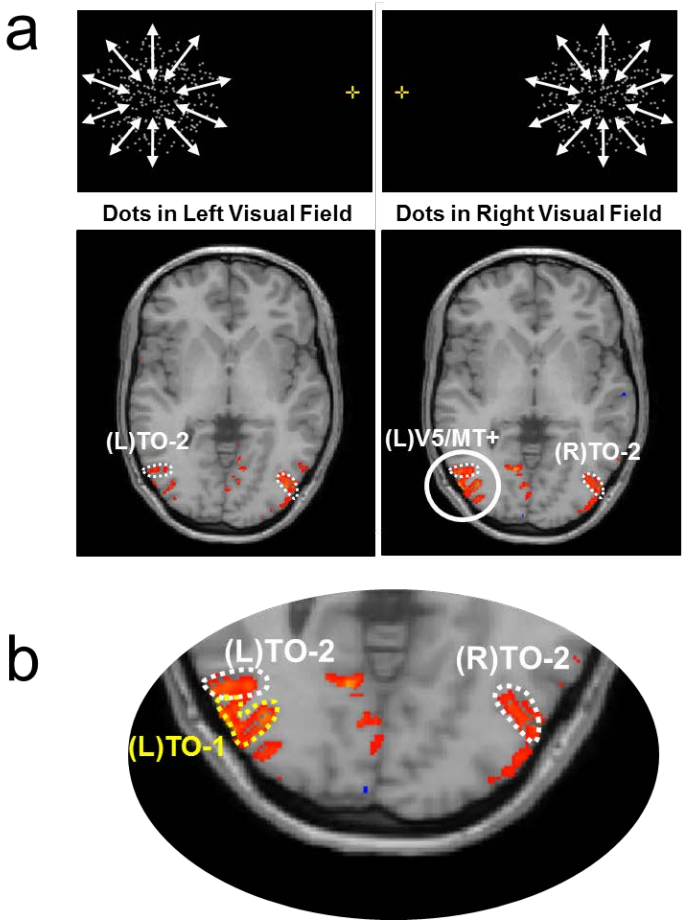

Figure 2.6. Stimulus specification and identification of TO-1 and TO-2. a) (Top Row) Example stimuli showing dots presented in either left or right visual field. (Bottom Row) Axial fMRI data from one representative subject (S3) showing BOLD signal ($p<0.001$) generated by moving vs static functional localisers presented in both left and right visual field (averaged across four runs). Anterior TO-2 (white dotted line) can be seen relative to whole V5/MT+ complex in both hemispheres and is identified as the only anterior portion of the complex activated by ipsilateral stimuli. b) Magnified view of posterior occipital lobe in the same subject when viewing dots in right visual field demonstrating the identification of TO-1. Here, left TO-1 (yellow dotted line) is shown as the subtraction of the TO-2 ipsilateral activation (white dotted line) from the whole V5/MT+ complex activated by contralateral stimulation.

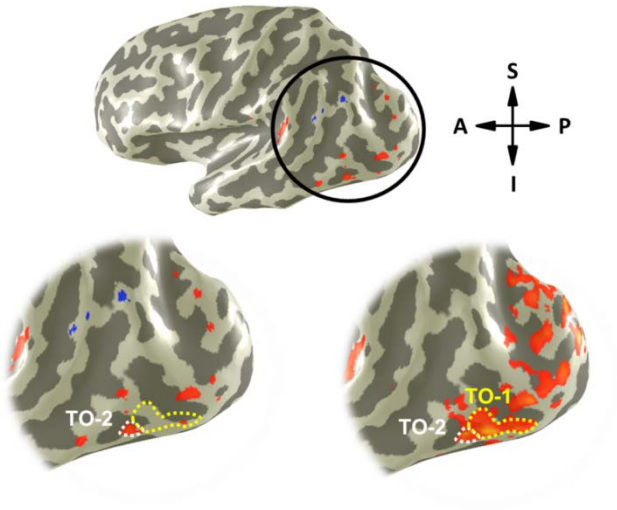

Figure 2.7. Areas TO-2 and TO-1 shown as increases in BOLD signal superimposed onto 3D inflated surfaces of the left cerebral hemisphere of subject S3. The black circle highlights the magnified area of the two images, with the left image showing ipsilateral activation of TO-2 (white dotted line) produced when dots were viewed in the left visual field. Similarly the right image shows identification of TO-1 (yellow dotted line) when TO-2 is subtracted from the full contralateral activation of V5/MT+ produced by viewing dots in the right visual field.

Although some local spread of the TMS magnetic field occurs across tissue adjacent to targeted sites, previous research has shown that the differential effects of TMS are measurable in target sites providing they are at least 10mm apart (Silson et al., 2013). Following this, 10mm was used as the minimum criteria for distance between target points in each subject. Target points for each of our sites of interest were created by overlaying the functional data onto a 3D structural scan and creating target points for both TO-1 and TO-2 based on their respective centres-of-mass. The Euclidean distance (d) between TO-1 (x_1, y_1, z_1) and TO-2 (x_2, y_2, z_2) target points were then computed using the following formula (Eq. 1):

$$d = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2 + (z_2 - z_1)^2} \quad (1)$$

It was found that TO-1 and TO-2 were at least 10mm apart in 11/14 hemispheres; however the only hemisphere of interest for this series of experiments was the right hemisphere (see Figure 2.8). One subject did not meet the minimum criteria for inter-target distance and also showed no effect on performance with TMS applied to either area, suggesting the two areas are not only close in proximity but also most likely deep within a sulcus. This subject (S5) was removed from the subset of subjects that were carried forward to take part in the TMS experiment.

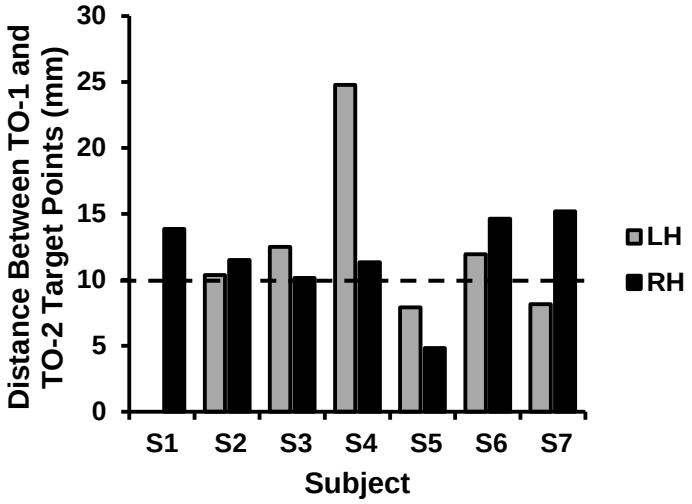

Figure 2.8. Bar chart showing Euclidean distance (mm) between TO-1 and TO-2 in both the left (LH) and right hemispheres (RH) for each subject. The horizontal black dotted line denotes the 10mm criterion. Some subjects fail to meet the criterion in their LH (S5, S7) or RH (S5) and so were removed from the sample. TO-2 was not reliably identified in the LH of S1.

2.5.2 Identification of Control Site (LO-1) and V3A

Retinotopic mapping techniques (Sereno et al., 1995; Engel et al., 1997) were used to identify V3A and the control site (LO-1) in each subject. Both

cortical regions of interest were successfully identified bilaterally in all subjects.

V3A was found to be located superiorly to V3d representing the inferior vertical meridian at the boundary (see Figure 2.9). In addition to this, LO-1 was found adjacent to V3d representing the contralateral lower visual field posteriorly, and the contralateral upper visual field anteriorly (see Figure 2.9). This is consistent with previous data pertaining to the topography of neuronal coverage within LO-1 (Larsson and Heeger, 2006; Silson et al., 2013).

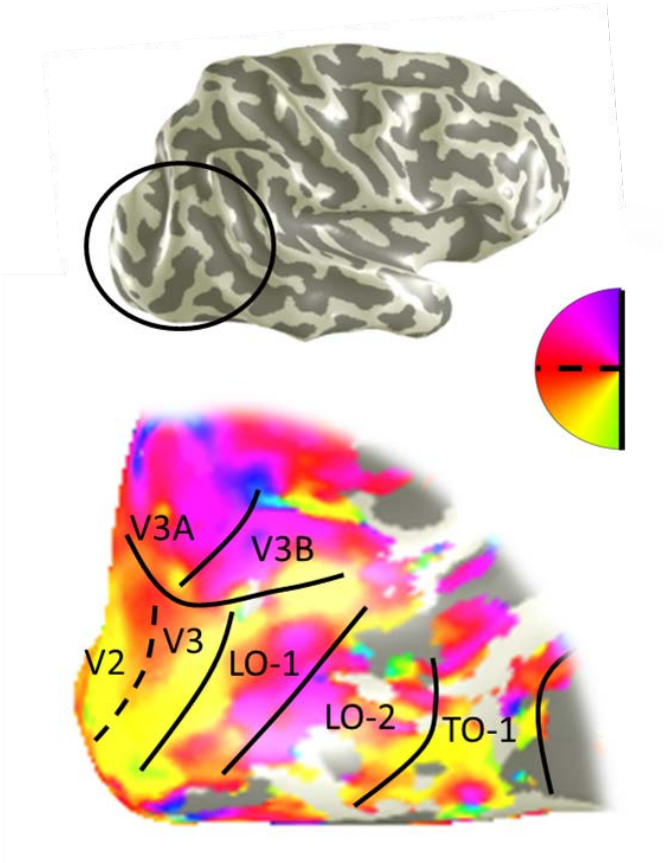

Figure 2.9. Figure demonstrating pseudo-colour maps of BOLD activity for retinotopic mapping on inflated right hemisphere of one sample subject (S3). The top image shows which part of the cortex the data is presented on, whilst the bottom image is a magnified view. This image demonstrates the representative pseudo-colour representation of the visual field (see key). Black dashed lines represent horizontal meridian, whilst solid black lines represent the vertical meridian. Identification of LO-1 and V3A was successful using this method.

Cortical area LO-1 was chosen as a control site because it lies in close proximity to areas TO-1, TO-2 and V3A, but unlike these areas, LO-1 has no known role in the processing of visual motion. Instead it appears to be involved in processing orientation information related to perception of objects (Larsson and Heeger, 2006; Silson et al., 2013). The use of this as a control site helped to determine whether there were any proximity effects of TMS on subjects' performance. It also allowed confirmation that any effects found from applying TMS to the target ROIs are not simply due to the general effect of applying TMS to the visual cortex.

2.5.3 Talairach Co-Ordinates

In order to confirm reliability of our findings, the Native MRI TO-1 and TO-2 target points were transformed into Talairach space. This allowed a direct comparison to be made between the mean co-ordinates of these target points with those reported previously. This data has been found to be largely consistent with previous research which suggests the TMS target points are appropriate (Table 2.1). The only slight difference exists in the z coordinates, as the coordinates reported for this experiment appear to be slightly lower which suggests a slightly more inferior representation than previously reported.

Table 2.1. Table comparing average Talairach co-ordinates (± S.D.) of TO-1 and TO-2 found in this study to previous studies for both the right and left hemispheres.

Right Hemisphere		**TO-1/MT**			**TO-2/MST**	
	x	*y*	*z*	*x*	*y*	*z*
This study	43 ± 3.4	-76 ± 3.1	-2 ± 7.7	45 ± 4.3	-70 ± 3.6	-1 ± 7.2
Dukelow et al,. 2000	44 ± 3	-64 ± 7	5 ± 4	45 ± 3	-60 ± 5	5 ± 4
Kolster et al., 2010	46	-78	6	44	-70	5
Left Hemisphere		**TO-1/MT**			**TO-2/MST**	
	x	*y*	*z*	*x*	*y*	*z*
This study	-45 ± 2.3	-78 ± 2.7	-2 ± 9	-46 ± 6.6	-69 ± 5.4	-2 ± 9.7
Kolster et al., 2010	-48	-75	8	-45	-67	6

2.6 Target Identification

Target sites for the TMS neuronavigation were computed by determining the centre-of-mass for each region of interest. These centre-of-mass co-ordinates were saved for each individual in BrainVoyagerQX in Native space (Table 2.2; Figure 2.10). Only target sites within the right hemisphere were used in these experiments.

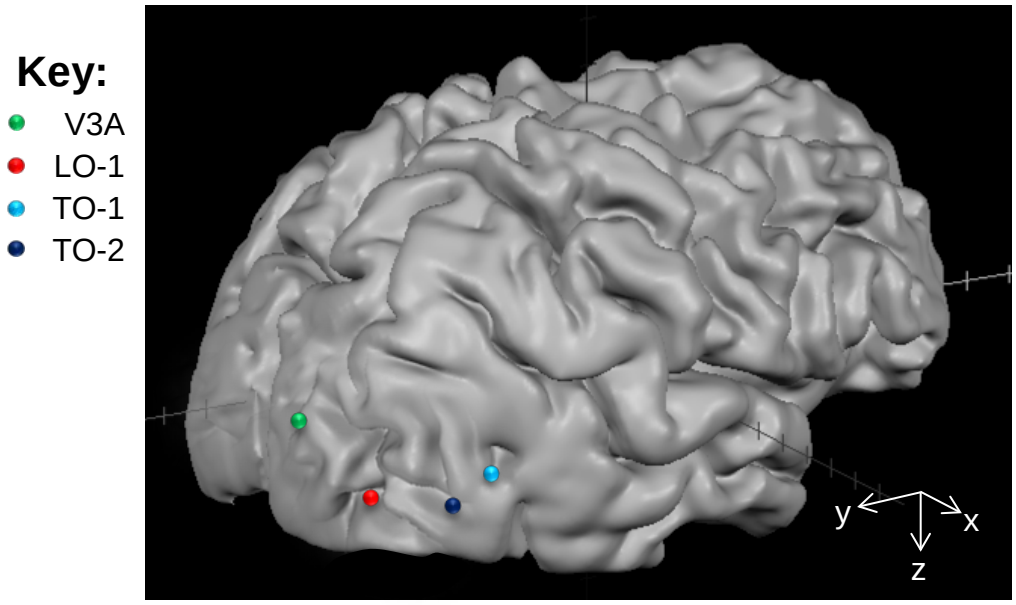

Figure 2.10. Schematic outlining relationship between TMS target points of S2. Figure created and adapted from Brainvoyager QX screenshot.

Table 2.2. Table outlining Native co-ordinates for all four TMS regions of interest (TO-1, TO-2, V3A, LO-1) in both the left (LH) and right hemisphere (RH).

RH	**TO-1**			**TO-2**			**V3A**			**LO-1**		
	x	*y*	*z*	*x*	*y*	*z*	*x*	*y*	*z*	*x*	*y*	*z*
S1	*198.9*	*141.8*	*88.1*	*186.3*	*145.1*	*83.4*	*217.7*	*126.1*	*118.2*	*212.4*	*146.5*	*107.4*
S2	*192.3*	*110.6*	*89.0*	*187.0*	*110.7*	*78.8*	*209.5*	*93.1*	*106.0*	*213.8*	*111.5*	*96.1*
S3	*212.4*	*117.4*	*88.7*	*209.3*	*109.2*	*83.6*	*222.0*	*96.0*	*123.4*	*227.4*	*122.6*	*102.6*
S4	*186.8*	*109.5*	*77.3*	*179.2*	*117.1*	*73.7*	*200.0*	*93.1*	*111.5*	*207.7*	*112.5*	*102.6*
S5	*185.5*	*136.6*	*77.3*	*183.2*	*132.6*	*75.9*	*203.4*	*117.9*	*105.7*	*209.6*	*138.6*	*103.6*
S6	*181.2*	*134.5*	*79.8*	*170.5*	*126.5*	*73.8*	*200.5*	*130.5*	*106.5*	*197.4*	*138.6*	*99.6*
S7	*185.8*	*141.2*	*81.7*	*175.0*	*132.2*	*87.3*	*205.4*	*118.7*	*115.5*	*208.1*	*137.5*	*105.8*
LH	**TO-1**			**TO-2**			**V3A**			**LO-1**		
	x	*y*	*z*	*x*	*y*	*z*	*x*	*y*	*z*	*x*	*y*	*z*
S1	-	-	-	-	-	-	*218.5*	*122.5*	*134.5*	*217.4*	*136.6*	*156.5*
S2	*202.4*	*107.4*	*175.2*	*194.4*	*102.8*	*170.5*	*216.1*	*90.3*	*140.7*	*219.0*	*114.5*	*156.2*
S3	*216.5*	*113.5*	*172.6*	*205.7*	*112.5*	*178.8*	*222.9*	*98.1*	*135.1*	*230.8*	*119.1*	*152.8*
S4	*186.4*	*122.5*	*177.4*	*168.7*	*108.4*	*187.5*	*203.0*	*98.1*	*148.0*	*211.2*	*115.4*	*140.2*
S5	*188.3*	*137.5*	*175.2*	*181.5*	*135.3*	*178.6*	*199.3*	*118.4*	*153.5*	*204.5*	*137.5*	*154.4*
S6	*182.8*	*141.5*	*170.8*	*173.1*	*139*	*177.3*	*199.7*	*121.5*	*140.6*	*193.2*	*140.5*	*160.1*
S7	*184.0*	*138.5*	*177.9*	*182.6*	*145.8*	*174.7*	*206.5*	*118.4*	*137.6*	*211.4*	*138.6*	*145.5*

2.7 Co-Registration between fMRI Scan and Subject's Head

Following identification of the target points in 3D virtual space, it is necessary to use a three-dimensional ultrasound digitizer CMS30P (Zebris) in conjunction with the BrainVoyager QX software to co-register the subject's head with the structural and functional MRI scans. This system creates a local spatial coordinate system which is able to link the spatial positions of the ultrasound transmitters with three pre-specified anatomical landmarks or fiducials (Figure 2.11).

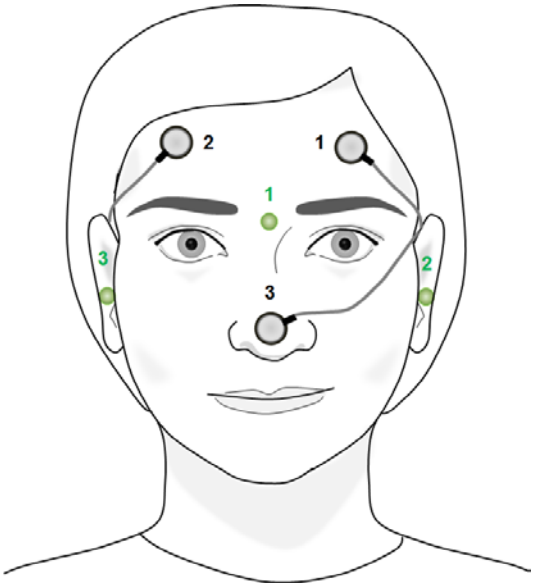

Figure 2.11. Schematic showing the relative positioning of the three ultrasound transmitters (grey) and the points identified by the digitizer pen (green).The position of the transmitters and the order of fiducials are crucial for maintaining an accurate representation of the head.

This is achieved using a digitizer pen which is able to send the real-time spatial co-ordinates of the three fiducials on the subject's head (nasion, both incisurae intertragicae) to the Zebris system (Figure 2.12).

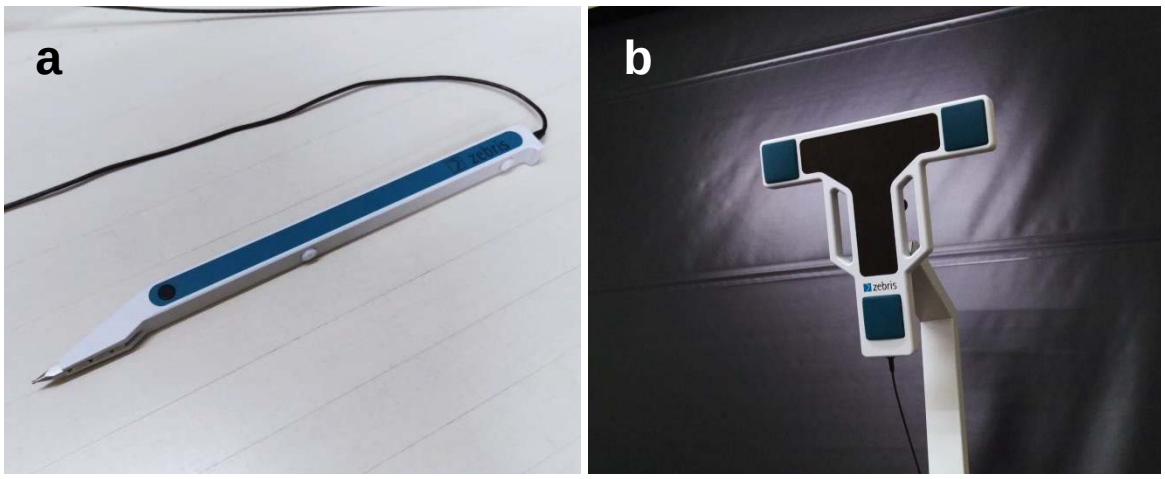

Figure 2.12. Photo of digitiser 'pen' (a) and Zebris CMS30P receiver (b) used to co-register head and coil with 3D representations.

This works by co-registering these real spatial co-ordinates with identically positioned pre-defined fiducials which are represented on the 3D head representation (mesh) of the participant in BrainVoyager. Using a similar method to co-register pre-specified points on the TMS coil with separate ultrasound transmitters, it is possible to visualise the location and movement of the coil in relation to the subject's head in real-time using BrainVoyager QX software (see Figure 2.13). Once this co-registration has taken place, the coil can be navigated over localised target ROIs and the position of the beam (in mm) can be monitored on a trial-by-trial basis. A total of four sites were located using this method, TO-1, TO-2, V3A, and LO-1 (control).

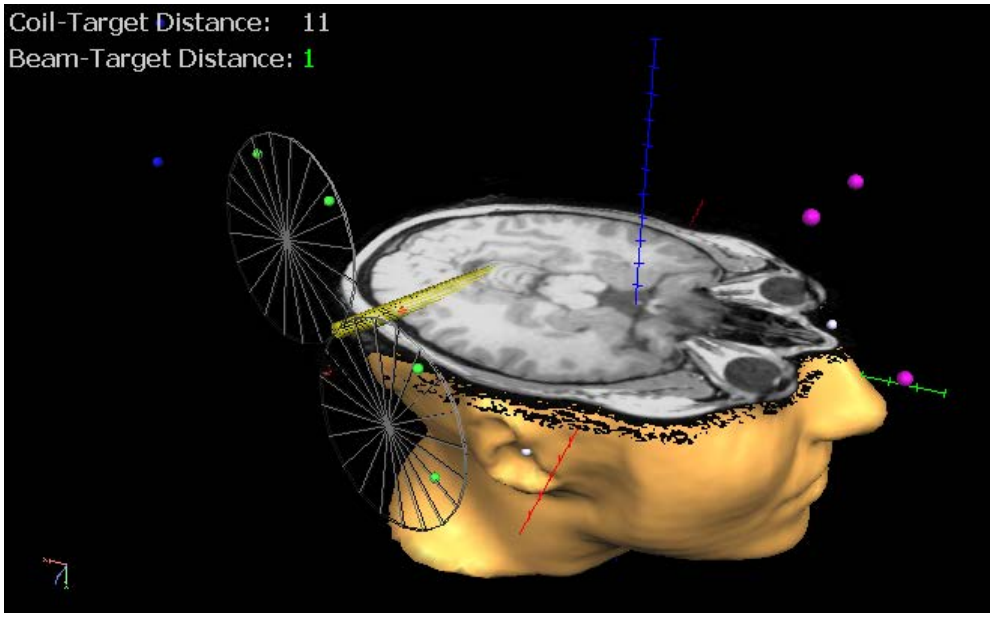

Figure 2.13. Screenshot of BrainVoyager QX software following co-registration of coil and subject. Red dot within cortex denotes target point (TO-1). Yellow beam denotes magnetic field of coils, and 'Beam-Target Distance' value is how the target site was confidently accessed. The pink dots show the relative position of the ultrasound transmitters, and the white dots indicate the position of the pre-determined fiducials.

2.8 General Transcranial Magnetic Stimulation Protocol

Transcranial magnetic stimulation (TMS) is a non-invasive technique that allows a localised, rapidly changing magnetic field to be delivered over the surface of the head which is achieved by transmitting a rapid current through an electromagnet (see Chapter 1; Section 1.4; Figure 1.7). This magnetic field has been shown to interfere with the normal electrical activity of neurons in the cortex by depolarising the cell membrane in the underlying cortex. The advantage of this technique is that the magnetic pulse is able to move freely through skin, muscle and bone without losing any strength or intensity. This enables it to stimulate the cortical neurons without the need for invasive procedures (Hovey et al., 2003). This interference can be conceptualised as a 'temporary lesion' which disrupts the normal function of cortical areas 1-2cm in diameter beneath the point of stimulation by contributing to neural noise (Hilgetag, 2001). This functional disruption can then be assessed using psychophysical methods to determine if the area of cortex is responsible for processing various attributes of vision.

This thesis utilised a variety of techniques including distal and repetitive online TMS; using a small 50mm figure-of-eight coil to investigate the functions of TO-1, TO-2 and V3A.

2.9 General Psychophysical Protocol and Stimuli

Stimuli were displayed on a high-resolution CRT monitor with a refresh rate of 75Hz (Mitsubishi DiamondPro 2070SB; active display area of 39.6 x 29.7 cm). Stimuli were generated using Psychophysics Toolbox Version 3

(Brainard, 1997; Pelli, 1997; Kleiner et al., 2007) in 32-Bit MATLAB (Version 7.6.0. Natick, Massachusetts: The MathWorks Inc., 2008). Subjects always viewed the monitor from a distance of 57cm (see Figure 2.14).

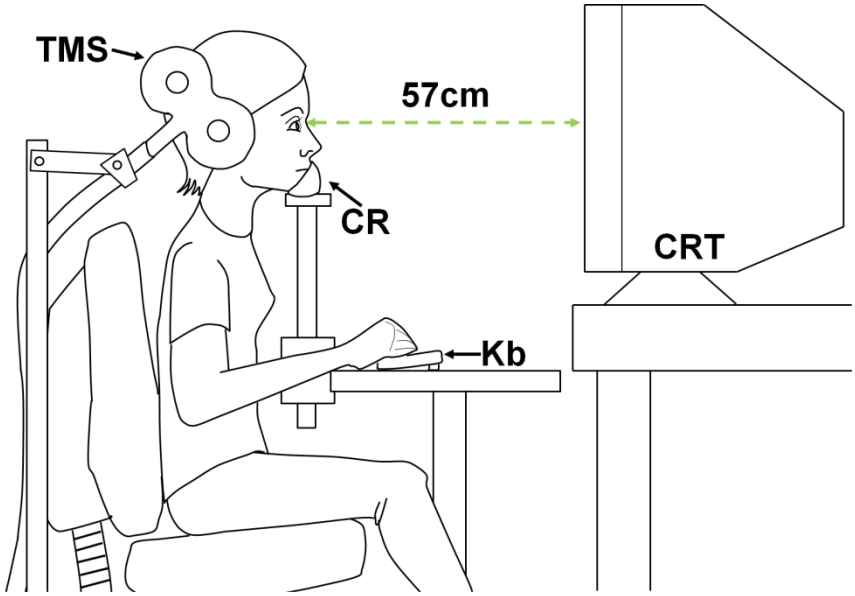

Figure 2.14. Schematic of typical experimental set up. Stimuli shown on CRT viewed at a distance of 57cm. TMS coil is positioned accurately using clamp. The subject places his/her chin in chin rest (CR) and makes responses using the keyboard (Kb).

In these experiments, subjects' ability to identify characteristics of moving stimuli was measured using psychophysical techniques. Measuring psychophysical ability is a commonly used technique for measuring behavioural performance of the perceptual system. In this instance the psychophysics employed measured visual motion perception.

There are many different types of psychophysical methods, including: method of constant stimuli, method of limits, method of adjustment, and forced choice, amongst others (Gescheider, 2013). The psychophysical procedure used by all experiments within this thesis was the method of

constant stimuli (Gescheider, 2013). This method involved sequential presentation of stimuli with difficulty levels ranging from very easy (100% correct) to a very difficult (0% correct). Importantly, the trials had to be varied randomly so that subjects would be unable to predict the outcome of each presented trial and subjects received no feedback as to how they were performing. This reduced any potential impact of bias because results were not affected by expectation or perceived success of the subject, thereby asserting a good degree of reliability.

Each difficulty level was repeated several times to allow the responses for each level to be averaged in order to ascertain the subject's performance with less impact of errors on the final result. The whole range produced a variety of responses that fell between 0% and 100% (see Figure 2.18). A psychometric function was then fitted to the data points and information was extrapolated from the curve i.e. difference thresholds.

These experiments also utilised a method of forced choice. This means subjects were required to make a decision regarding the stimulus against one of two options. The subject's threshold level would be equivalent to the point at which the subject started to be able to detect a difference between the two stimuli. In these experiments, 75% was used because 50% correct would only equate to a chance level of performance ability. This meant our usable data was likely to entirely exist between values of 50%-100% and so the threshold level was adapted to 75% in order to account for this.

2.9.1 Choice of Stimuli

In order to successfully design psychophysical experiments that identify functional differences between TO-1 and TO-2 it was necessary to review both non-human primate and human neuroimaging literature to identify an appropriate task.

It has already been described in Chapter 1 that one of the most consistently identified functional differences between MT and MST in non-human primates exists between translational, radial and rotational motion directions (Saito et al., 1986; Duffy and Wurtz, 1991a; Duffy and Wurtz, 1991b). Translational directions constitute 'simple' motion, whereas radial and rotational directions are often termed 'complex' motion as they contribute to analysis of optic flow (Huk et al., 2002). In non-human primates, MT is often associated with processing of translational motion whilst MST is more commonly associated with radial and rotational motion (Saito et al., 1986). This led further researchers to investigate whether the same distinction is evident between TO-1 and TO-2 in humans using neuroimaging techniques (Smith et al., 2006; Wall et al., 2008; Pitzalis et al., 2013c). These experiments found indications of functional differences between TO-1 and TO-2, particularly between expanding (radial) and translating stimuli. However, as of yet there has been no causal evidence to suggest functional differences exist between these areas within the human brain, and as such, this thesis aimed to use TMS to allow conclusions to be drawn regarding causality. In order for the experiments described here to be comparable to previous work, the stimulus parameters needed to be designed to be in line

with what would be expected of MT and MST in non-human primates and with tentative findings from human neuroimaging studies. This meant the most appropriate measure would be motion coherence-defined direction discrimination along three motion domains (Figure 2.15): translational (upwards/downwards), radial (outwards/inwards) and rotational (counter-/clockwise).

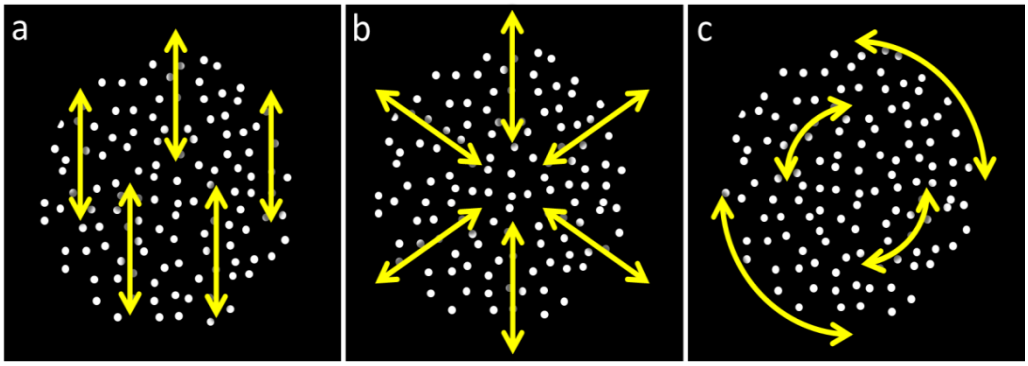

Figure 2.15. Demonstration of three motion domains tested throughout this thesis: translational (a), radial (b), and rotational (c).

Movement of the dots was determined using mathematical formulas to plot trajectories according to the direction required: translational (Eq. 2a), radial (Eq. 2b), and rotational (Eq. 2c).

$$\frac{dy}{dt} = v \sin \alpha \tag{2a}$$

$$\frac{dr}{dt} = v \cos \phi \tag{2b}$$

$$\frac{d\theta}{dt} = (v \sin \phi)/\mathrm{r} \tag{2c}$$

In these equations (2a-c), v represents local speed (°/s), α represents direction of motion, θ represents the angle between the x axis and the radial

position, ϕ symbolises the angle defining optic flow trajectory, and r equates to the radius. If the value of ϕ is equal to $\pm\pi/2$ the trajectory of the dots will be rotational (clockwise or anticlockwise). It should be noted that this equation is divided by *r* in this case to ensure all dots move at the same speed despite being different distances away from the centre of motion, as this ensures the rotational motion task is comparable to the translational and radial tasks. In contrast to this, if ϕ is equal to 0 or π, the trajectory of the dots will be radial (outward or inward). The centre of the radial and rotational motion was always set to be located at the centre of the presentation aperture. These equations are visualised in Figure 2.16 below.

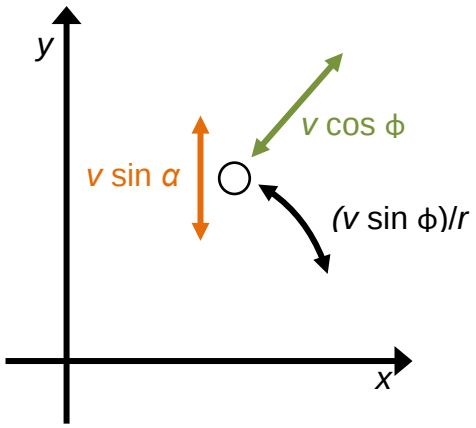

Figure 2.16. Schematic outlining plotted trajectories of dots for translational, radial and rotational motion.

The specific parameters of these stimuli were determined in preliminary experiments described below.

2.10 Preliminary Psychophysical Functions: Identifying Threshold Level Performance

2.10.1 Introduction

The first preliminary experiment sought to investigate whether the three tasks were behaviourally possible, and if so, what an appropriate range of coherences would be for each type of motion (translational, radial, rotational), as potentially one of the directions may be perceptually easier to identify than others (Freeman and Harris, 1992). This involved testing ability of subjects on each task in baseline (no TMS) conditions across various ranges of coherence levels.

2.10.2 Methods

2.10.2.1 Subjects

Seven subjects took part in this experiment (mean age, 29.9 years; range 21-46 years; three female). All subjects were recruited from the University of Bradford and the University of York and all had normal or corrected-to-normal vision at the time of testing. No subjects had any history of neurological or psychiatric disorders.

2.10.2.2 Stimuli and Psychophysical Procedure

The stimuli consisted of 300 white dots (size: ~0.2°; density: 4.69/deg^2) presented on a black background. These dots appeared within a 10° aperture, the closest edge of which being positioned 10° left of fixation

(Figure 2.17). Dots moved at a speed of 7°/s and were presented for 200ms on each trial. On every trial a percentage of dots would move in a coherent direction (signal dots) in one of three motion domains: translational (upwards/downwards), radial (outwards/inwards), rotational (counter-/clockwise). This percentage of coherence was assigned randomly on each trial from one of seven possible coherences across a pre-determined range of percentages (max 50%). The remaining percentage of dots moved in random directions (noise dots).

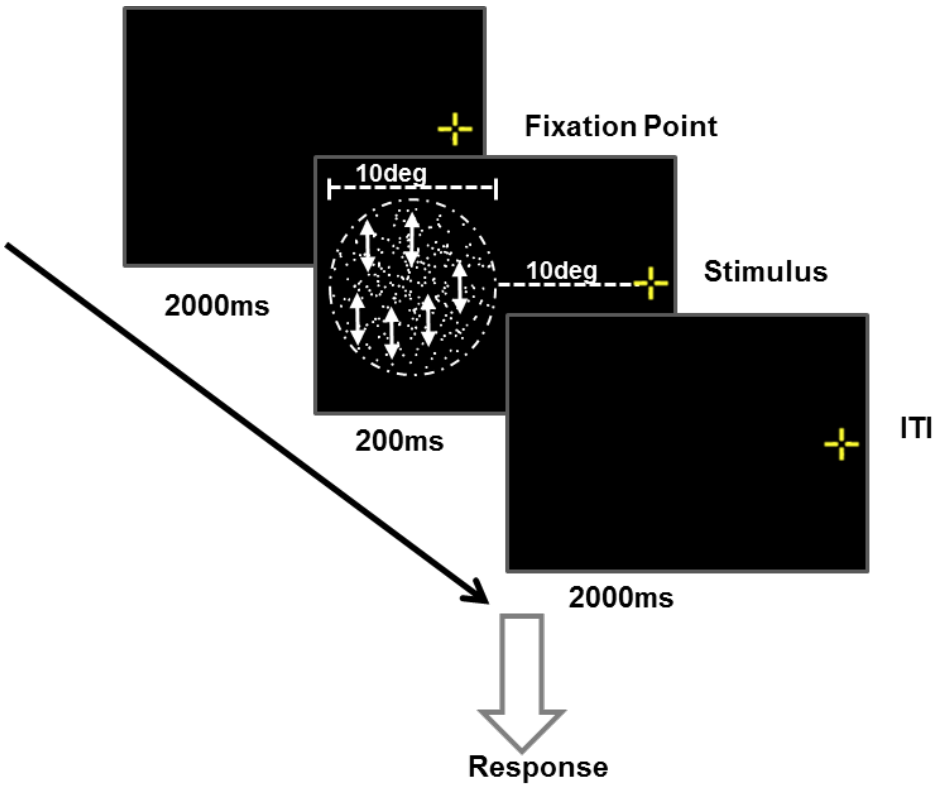

Figure 2.17. Schematic outlining psychophysical procedure of this experiment using translational motion as the example direction domain.

Subjects saw 25 presentations of each coherence level for each motion type (total 175 trials per task) and sat at a viewing distance of 57cm (left eye

occluded).The range of coherences varied depending on the subjects' ability to perform on each task, as an inappropriate range could have produced inaccurate threshold levels in the analysis.

2.10.2.3 Fitting the Psychometric Functions

Once subjects completed a condition, their performance was plotted. An example is shown in Figure 2.18. This plotted the average values for performance against each particular coherence level. It is clear therefore that performance begins on the left at ~50% correct when the task is difficult (chance performance), and as the percentage of coherence (signal) increases, the performance also increases up to ~100% correct.

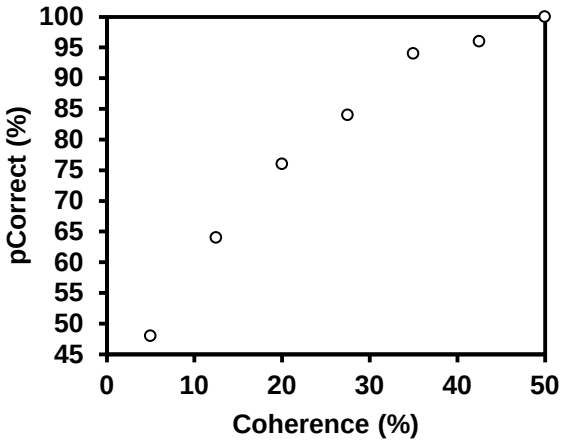

Figure 2.18. Figure showing example (S4) raw psychophysical data.

The data points were then fitted by a two-parameter logistic function taking the form of (Eq. 3):

$$P(x) = [1 + \exp\{\delta(\theta - x)\}]^{-1} \quad (3)$$

In this case, x represents the stimulus value (coherence), $P(x)$ is the response probability at x, δ is the slope parameter, and θ represents the threshold parameter corresponding to the stimulus level at which response probability is 75%. In order to successfully fit this function, it was first necessary to estimate starting parameters for δ and θ so that a non-linear regression could be applied and more accurate parameters could be deduced. These initial starting estimates were achieved by expressing the raw response probabilities as natural log values in order to achieve a full range from 0-1. This formula (Eq. 4) required:

$$logit[P(x)] = \ln\left[\frac{P(x)}{1 - P(x)}\right] \tag{4}$$

Now plotting $logit[P(x)]$ against x is a linear function. This linearity was then assessed regarding whether it was a good fit or not (R^2*>0.8*), and if it was found to be a good fit, it justified the use of the logistic function, and it also produced useful starting estimates. These estimates were equated by using the equation (Eq. 5) for the linear function of $logit[P(x)]$ against x.

$$logit[P(x)] = mx + c \tag{5}$$

In this instance, the m value represents the slope (δ) estimate and the x value equated to our estimate of threshold (θ). Using this formula x was computed by working out what value would be necessary for the $logit[P(x)]$ value to equate to 0.0. This was done by Equation 6:

$$x = \frac{-c}{m} = 0 \tag{6}$$

These slope and threshold parameter estimates were then substituted into the original logistic and it was possible to perform a nonlinear regression using statistical software (IBM SPSS Statistics 20). This nonlinear regression provided the final parameter estimates for further analysis, along with the corresponding confidence intervals. It also provided the appropriate predicted values for plotting the psychometric function (see Figure 2.19). These threshold values were recorded and compared across subjects for each task.

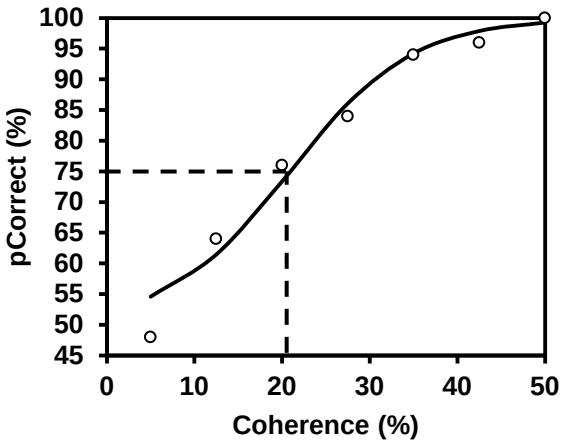

Figure 2.19. Figure showing psychometric function applied to example data (S4). The point at which the 75% performance horizontal line intersects the function is the point at which both threshold and slope values are extracted.

2.10.3 Results

2.10.3.1 Overall Performance

Across the three motion tasks, overall performance varied both between and within subjects (Figure 2.20). This shows that individual ability can differ between subjects, but also that the difficulty of the task is not entirely comparable across the three types of motion. For example, in all seven

individual plots, the rotational (green) data is further left than the translational (black) data, and in six of the plots it is further left than the radial (blue data). This resulted in lower thresholds for rotational motion which meant the task must have been perceptually easier.

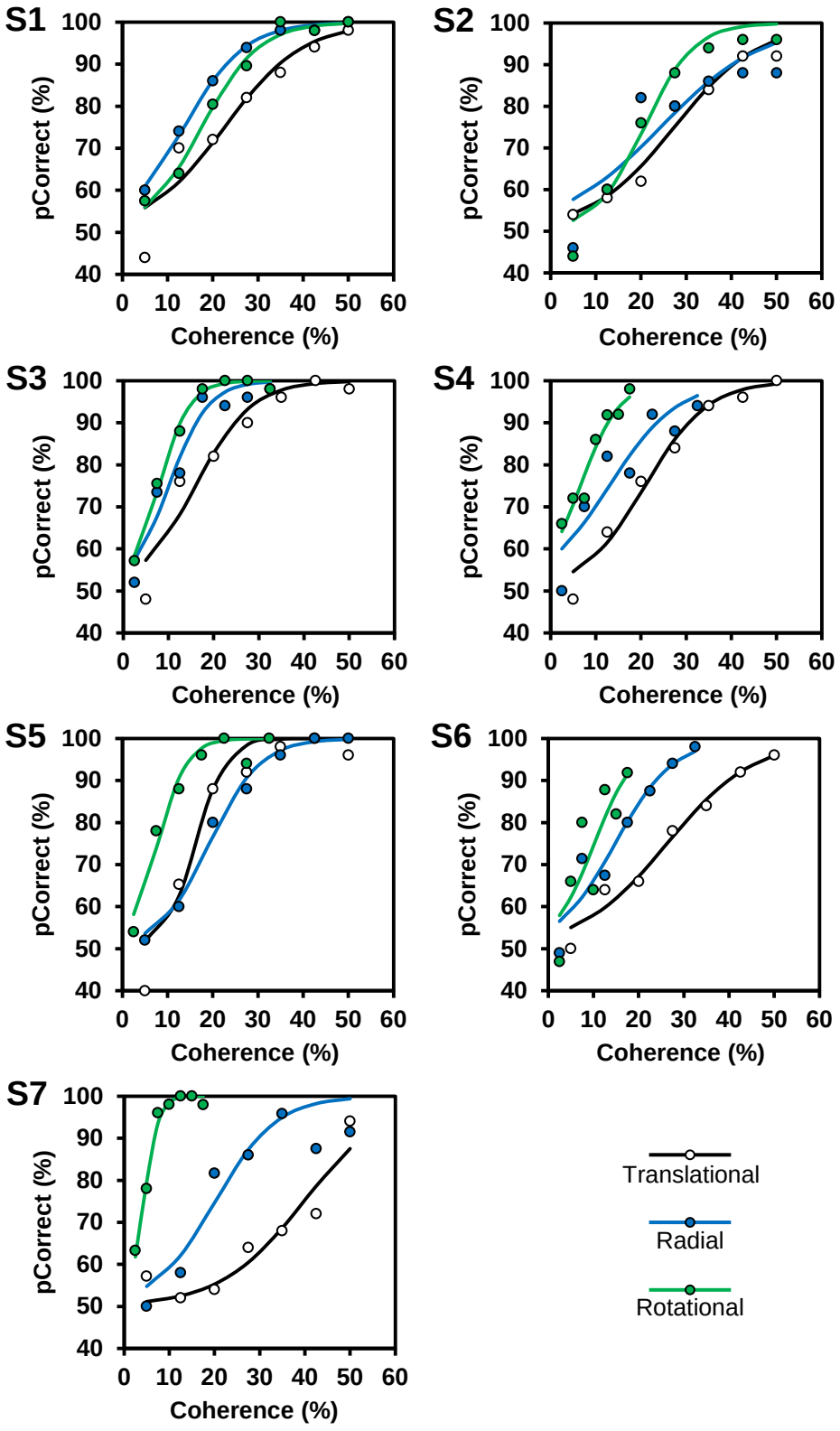

Figure 2.20. Plots showing individual psychometric functions (seven subjects). Performance is plotted as a function of motion coherence for translational (black line), radial (blue line) and rotational (green line) tasks. Not all tasks were performed across same coherence ranges between subjects.

2.10.3.2 Extracted Threshold Levels

From the psychometric functions, the 75% threshold for each individual (θ) could be extracted using the formula described above (Section 2.10.2.3). These baseline (no TMS) thresholds varied greatly between subjects and but more so across motion tasks (see Figure 2.21; Table 2.3).

Statistical analysis of this data was performed once Mauchly's Test of Sphericity confirmed assumptions of normality were met ($\chi^2(2) = 2.76$, $p = 0.251$). A repeated-measures ANOVA highlighted a significant main effect of motion task on threshold ($F(2,12) = 15.56$, $p < 0.001$). Subsequent pairwise comparisons (Bonferroni corrected) demonstrated that this observed effect was the result of significant differences between Translational and Radial ($p = 0.014$), and Translational versus Rotational ($p = 0.019$). Comparisons between Radial and Rotational did not produce significant differences ($p = 0.086$). As low thresholds indicate a better performance, this confirms that both the radial and rotational tasks were easier than the translational task.

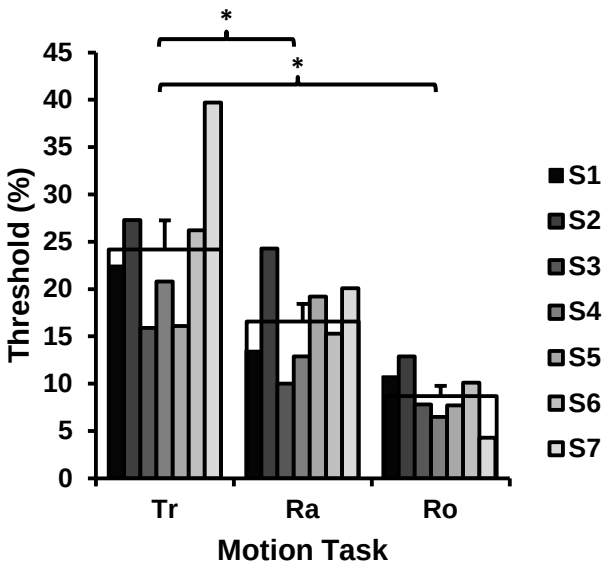

Figure 2.21. Bar chart showing individual 75% thresholds for translational (Tr), radial (Ra) and rotational (Ro) moving dot patterns for each subject. Average values are overlaid across each of the tasks. Error bars represent S.E.M. Asterisks highlight significant differences at $p = 0.05$ level.

For reference, the individual threshold values are outlined in the table below (Table 2.3).

Table 2.3. Table showing individual 75% coherence thresholds for all three types of moving dot pattern for each subject. Table also demonstrates average values ± standard deviation.

Subject	Translational (%)	Radial (%)	Rotational (%)
S1	22.6	13.6	10.9
S2	27.4	24.4	13
S3	16.0	10.1	7.9
S4	20.9	13.0	6.6
S5	16.2	19.3	7.8
S6	26.3	15.4	10.2
S7	39.8	20.2	4.4
Average	*24.2 ± 8.2*	*16.6 ± 5.0*	*8.7 ± 2.9*

2.10.4 Discussion

This technique successfully identified threshold levels for each subject for each type of motion (translational, radial, rotational). However, it did uncover significant differences between motion tasks, specifically between translational motion and both radial and rotational motion (Figure 2.21). This can be interpreted as showing that on average; both the radial and rotational tasks were perceptually simpler than the translational task, a result which is consistent with findings from earlier psychophysical experiments (Freeman and Harris, 1992). This assumption is further supported in the need for smaller ranges of coherences (2.5-17.5%) for some subjects during the rotational task, compared to a consistently required larger range of coherences (5-50%) for the translational task. This shows that subjects required fewer signal dots for radial and rotational motion in order to reach threshold-level performance. Finding this difference suggests that caution will need to be taken in future experiments when attempting to compare performances across tasks as they are not equivalent at baseline. It also highlights that it is not necessarily appropriate to attribute the identical coherence ranges to every subject or task. The experiments will need to be designed to account for these individual differences in performance between each other and across tasks (Figure 3.4).

In summary, the radial and rotational tasks were perceptually easier than the translational task and performance varied across subjects on all three tasks.

2.11 Investigating Effects of Dot Density on Baseline Performance

2.11.1 Overview

Previous research has proposed that motion sensitive areas in the visual brain may show preferences for varying dot densities (Braddick et al., 2001) i.e. proportion of dots per visual angle. For example, within areas that demonstrate large neuronal receptive fields, Braddick et al. proposed that a low density of dots may be unlikely to produce optimal activation and that instead, a higher density of dots may induce stronger effects. However, previous psychophysical experiments have demonstrated that dot density has little effect on thresholds (Barlow and Tripathy, 1997) and more recent experiments investigating differences in stimuli and the relative effect on activation within hV5/MT+ also report no effect of dot density (Becker et al., 2008). This coincides with previous results from non-human primates showing that changes in dot density have little effect on the responsivity of neurons within MSTd (Duffy and Wurtz, 1991a). More specifically, Becker et al. (2008) propose that providing the size of the dot display (aperture) is larger than 8°x8°, this will produce optimal activation in hV5/MT+ as it is large enough to account for the larger receptive fields. However, these researchers only measured the effect of dot density on a stimulus display size smaller than they proposed would be appropriate for optimal activations within hV5/MT+ (5.3°). This then leads to the question of whether using a larger more appropriate display (10°) will lead to differences in the effect of dot density.

2.11.2 Hypothesis and Aims

This experiment aimed to investigate potential behavioural differences between dot densities ranging from 2 /deg^2 – 10 /deg^2 within an aperture that would be appropriately sized for activating hV5/MT+ (10°). It was hypothesised that performance will remain consistent across all dot densities.

2.11.3 Methods

2.11.3.1 Subjects

Five subjects took part in this experiment (two male; mean age 27.4; age range 21-35). Four of these subjects (two female) were naïve to the aims of the experiment (S4, S5, S6, S7) and all experimental procedures were single-blind. All subjects had normal or corrected-to-normal vision at the time of testing and had no history of neurological or psychiatric disorders.

2.11.3.2 Psychophysical Stimuli and Procedure

Stimuli comprised a 10° aperture of a varying number of dots (speed: 7°/s; size: ~0.2°). Subjects participated in a two-alternate-forced-choice (2AFC) procedure in which they were sequentially shown a randomised percentage of coherent dots moving either upwards or downwards within a field of randomly moving dots exactly as described for the translational task in the previous experiment (Section 2.10.2.2). This percentage of coherent dots

ranged from 5%-50% in steps of 5. Each condition involved a different number of total dots within the presentation aperture depending on the tested dot density (range 2-10, see Figure 2.22).

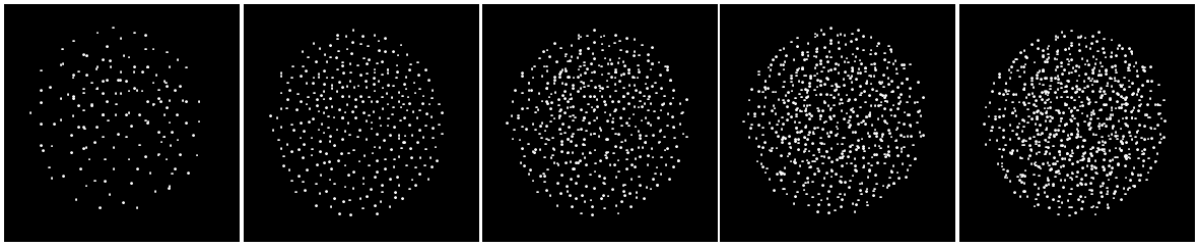

Figure 2.22. Demonstration of five possible dot densities (DD). For this experiment, dot values were: DD2 (158 dots), DD4 (314 dots), DD6 (472 dots), DD8 (628 dots), DD10 (786 dots).

The total number of dots ($tDots$) required for each dot density was computed using Equation 7:

$$tDots = DD \times (\pi r^2) \tag{7}$$

Where DD represents dot density, and πr^2 represents the area of the aperture. Wherever a total number of dots did not equate to an even integer, it was rounded up/down appropriately.

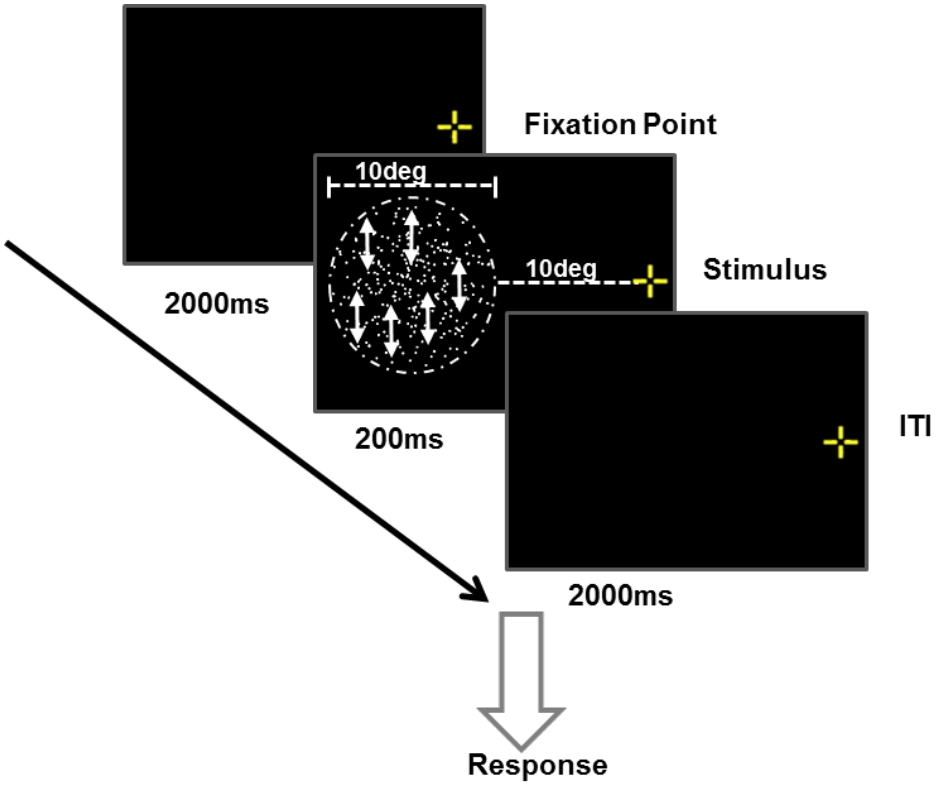

Figure 2.23. Demonstration of psychophysical procedure. Stimuli were presented for 200ms and subjects responded in the inter-trial interval (ITI) immediately following cessation of dots.

Subjects sat at a viewing distance of 57cm and responded using arrow keys on a computer keyboard (Figure 2.23). Each subject saw 25 repeats of each coherence level for each density (total 175 trials per condition). Once the data was collected, each individual dataset was fitted with a psychometric function as described previously (Section 2.10.2.3). The 75% threshold values were then extracted and compared across conditions and subjects.

2.11.4 Results

Average threshold data for each dot density was analysed following completion of the experiment (Figure 2.24). Mauchly's test of sphericity showed that the data did not violate assumptions ($\chi^2(9) = 4.32$, $p = 0.920$),

and a subsequent repeated-measures ANOVA highlighted no significant effect of dot density on thresholds for translational motion ($F(4,16) = 2.45$, $p = 0.089$).

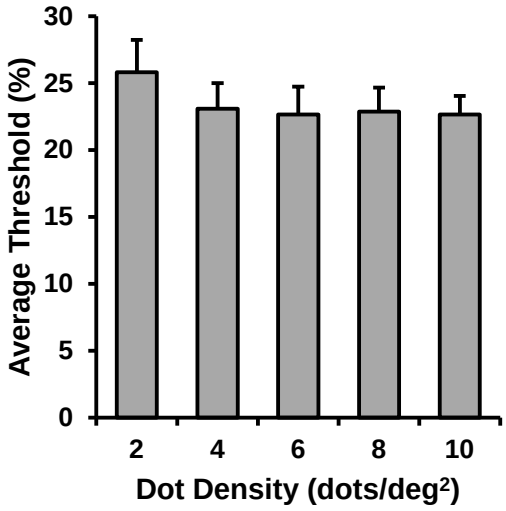

Figure 2.24. Bar chart displaying average threshold (%) values for each of the five dot density conditions (2-10). Error bars represent S.E.M.

2.11.5 Discussion

These non-significant results show that a broad range of dot density values ($2/deg^2$ - $10/deg^2$) all produce equivalent behavioural performances. This is shown by the similarities between average threshold values across each dot density level. This suggests that providing subjects have normal function of TO-1 and TO-2, dot density is not a variable that will affect performance on translational motion coherence direction discrimination tasks.

This coincides with previous literature outlining that changes in density of dots does not alter functional characteristics of V5/MT+ within the human brain (Becker et al., 2008) or affect firing rate of the neurons within MSTd in

the non-human primate brain (Duffy and Wurtz, 1991a). However as no TMS has been applied in this experiment, it is difficult to accurately conclude whether dot density has an impact on processing within TO-1 or TO-2. Rather, these results suggest that there is no natural perceptual advantage for one particular dot density over another and therefore any future experiments should not need to consider dot density a risk for confounding measured variables.

Instead, this data suggests that providing the dot density remains consistent across tasks and conditions, subjects should produce appropriately comparable performances.

In conclusion, altering the dot density across a broad range of total dots (158-762) during translational direction discrimination tasks does not lead to differences in ability of the subjects. This indicates that dot density should not need to be an important consideration for future experiments.

2.12 Investigating the Effect of Varying Strength of TMS on Performance on a Direction Discrimination Task

2.12.1 Overview

This experiment served to facilitate the design of the following TMS experiments by investigating the effect of applying varying strengths of TMS during the subject's performance on a translational motion task. This is the task previous literature posited would be most likely to be processed by visual area TO-1 (Smith et al., 2006; Wall et al., 2008; Amano et al., 2009), which means it is likely to show an effect that will assist with determining the most appropriate TMS protocol. It was not important however for this experiment to find a difference in function between TO-1 and TO-2, so the only tested experimental site was TO-1.

This experiment also sought to ascertain whether there was a relationship between the strength of the TMS and the effect on performance i.e. does the strength of the TMS correlate with subjects' performance on a direction discrimination task.

A control site was still tested however, in order to ensure that the stronger application of TMS was still not confounding any genuine effects of disrupting cortical activity within motion-sensitive areas. A good control site would be LO-1, as it is close to TO-1 in proximity but comprises object-sensitive, not motion-sensitive cortex (Malach et al., 1995; Larsson and Heeger, 2006). The final aim therefore was to discern whether LO-1 would serve as an appropriate TMS control site.

2.12.2 Hypothesis and Aims

It was hypothesised that application of TMS to TO-1 would produce a measurable reduction in performance on the translational motion task, and that stronger (higher percent strength) TMS pulses would produce larger effects than weak TMS pulses. It was predicted therefore that there would be a negative correlation between performance and TMS strength.

The final hypothesis posited that application of TMS to LO-1 would not produce any deficit in performance.

2.12.3 Methods

2.12.3.1 Subjects

Two of the seven subjects (S1, S3) participated in this experiment (mean age 33.5, one female). Both subjects had normal or corrected to normal vision at the time of testing and had no history of neurological or psychiatric disorders.

2.12.3.2 Threshold Identification

For this task, individual 75% threshold values were extracted from baseline translational data in the previous experiment (see Table 2.3: S1 22.6%; S3 16.0%). These values were then super-imposed into new individualised experimental MATLAB codes so that each subject had a tailor-made experimental protocol according to ability.

2.12.3.3 Target Sites

TMS Target sites were identified for TO-1 and LO-1 using co-ordinates outlined in Section 2.6.

2.12.3.4 Psychophysical Procedure

Subjects were required to undertake a similar motion-coherence task to the one described in Section 2.10.2.2 using the same stimulus parameters. A 2AFC paradigm for just one of the three types of motion: translational. On each run, the percentage of coherently moving dots was matched to the subjects' individual threshold value, and each run consisted of 25 presentations of this level (~1 min duration). Subjects were required to undertake four runs for each condition which totalled 100 trials per condition.

Subjects viewed a centrally placed fixation cross with their right eye (Figure 2.25) from a distance of 57cm, with the test stimuli being positioned 10° to the left of the fixation point. Subjects recorded their response with a key press.

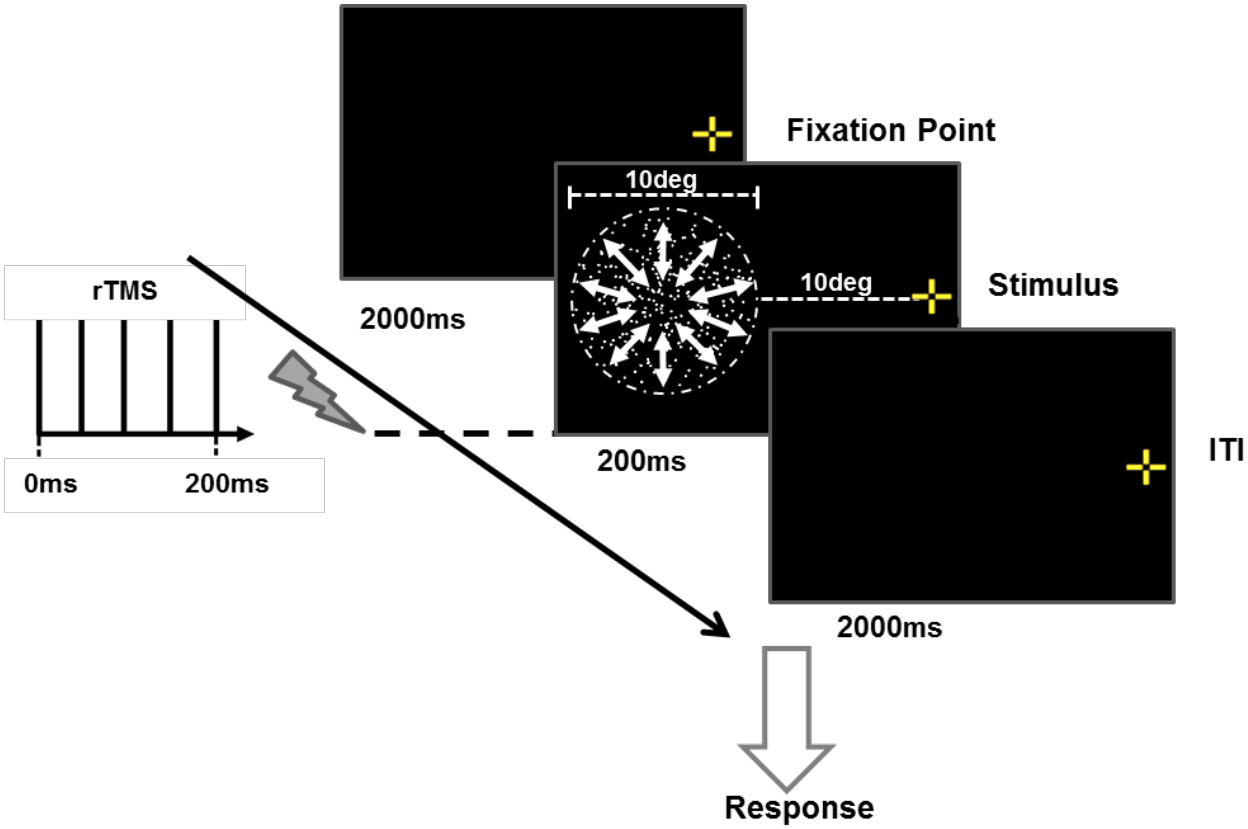

Figure 2.25. Schematic of psychophysical procedure. There is a 2000ms ITI, in which the subject is required to make a response. The stimulus is shown for 200ms and onset of TMS and stimuli are synchronous. Strength (%) of TMS varies across conditions.

2.12.3.5 TMS Protocol

Using the psychophysical method as described above for target site TO-1, this experiment examined the effects of online TMS at varying strengths from 45%-70% in steps of 5%. This involved applying TMS at a frequency of 25Hz synchronous with stimulus presentation (200ms duration; see Figure 2.25). This TMS was applied concurrently with onset of the stimulus for each trial. For the control site (LO-1), only 70% strength was tested. All measurements of performance recorded during these experimental conditions were compared to a 'no TMS' baseline. During application, the coil was secured in

a mechanical clamp and its position relative to the target ROI was monitored in real-time throughout the delivery of the pulses.

Performance on the task (% correct) and response times (s) were recorded for each TMS strength.

2.12.4 Results

2.12.4.1 Performance on the Task

In conditions where TMS was applied to TO-1, a steady decrease in ability was observed across both subjects (see Figure 2.26). However, when TMS was applied to the control site (LO-1) at the highest tested strength, the performance remained consistent with baseline (no TMS) performance.

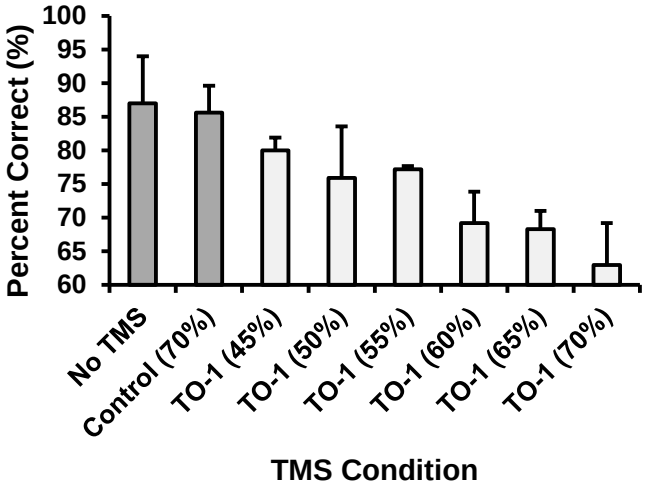

Figure 2.26. Bar chart showing average percent correct for the two subjects. Dark grey bars show baseline (no TMS) and control (TMS applied to LO-1) conditions. Light grey bars show performance when varying strengths of TMS are applied to TO-1. Error bars represent S.E.M.

A paired samples t-test showed only one significant difference from the no TMS condition; when TMS was at 55% strength ($t(1)= 14.31$, $p = 0.044$). No

other comparisons reached significance (45%, t(1)=2.33, p = 0.258; 50%, t(1)=2.17, p = 0.274; 60%, t(1)=2.74, p = 0.223; 65%, t(1)=7.93, p = 0.080; 70%, t(1)=5.63, p = 0.112). Fortunately, paired samples t-tests showed no significant difference when TMS was applied to the control site LO-1 (t(1)=1.80, p = 0.323).

2.12.4.2 Relationship between TMS Strength and Performance

Table 2.4. Table reporting means and standard deviations for decrease in percent correct and increase in response time.

	Mean	Std. Deviation
Decrease in pCorrect	-14.76	6.47
Increase in Response Time	0.55	0.28

A Pearson's r correlation coefficient was computed to assess the relationship between the strength of TMS applied to TO-1 and the average performance (percent correct) on the translational task (Table 2.4). This analysis shows a strong negative correlation between the two variables (r = -0.96, n = 6, p = 0.002). For this correlational analysis, the 'no TMS' condition was removed due to the unequal distance between 0% and 45% compared to the other conditions. Individual data outlining the linear relationship between strength o TMS and performance are shown in Figure 2.27. A scatterplot summarises the average relationship in Figure 2.28. In this figure, the percent correct (pCorrect) is plotted as the relative decrease from the no TMS condition, so 0 would be equivalent to average baseline performance. A negative relationship therefore demonstrates attenuated performance.

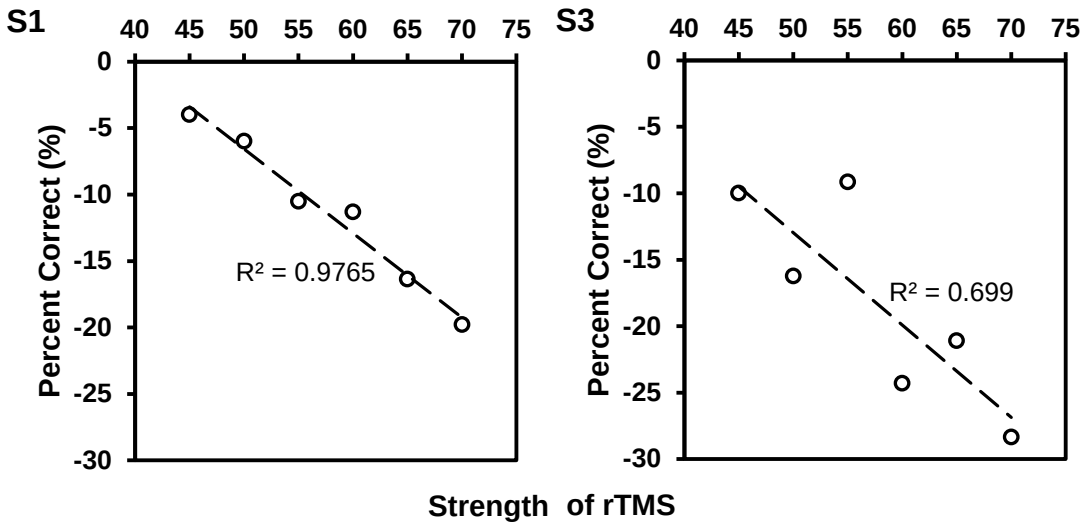

Figure 2.27. Individual plots showing decrease in percent correct values relative to baseline (no TMS) performance for the two subjects (S1, left; S3, right) when TMS is applied to TO-1. Black dashed line denotes linear trendline (R^2 is displayed on each chart).

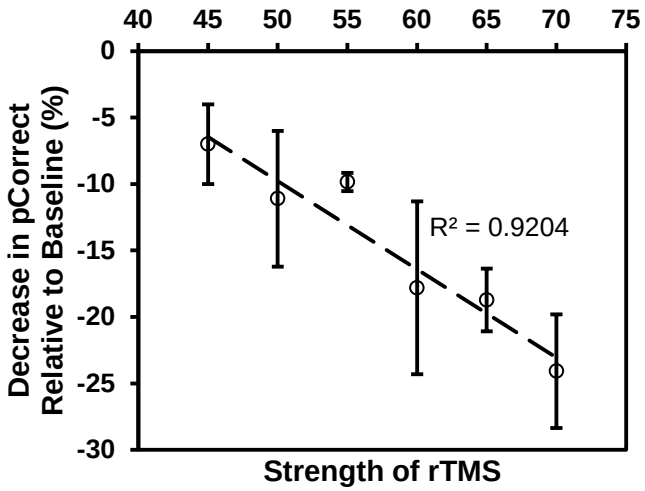

Figure 2.28. Scatter plot showing average decrease in % correct values relative to baseline (no TMS) performance for the two subjects when TMS is applied to TO-1. Black dashed line denotes linear trendline. Error bars represent S.E.M.

A Pearson's R correlation coefficient applied to the average response time results shows a strong positive correlation between the strength of TMS and

the time it takes for a subject to respond in the experimental condition (r = 0.85, n = 6, p = 0.031). Again, the 'no TMS' condition was removed for this analysis due to the unequal distance between 0% and 45% compared to the other conditions. These results are summarised in the scatterplot in Figure 2.29, where the increase in response time (RT) is plotted as the increase relative to the baseline (no TMS) condition.

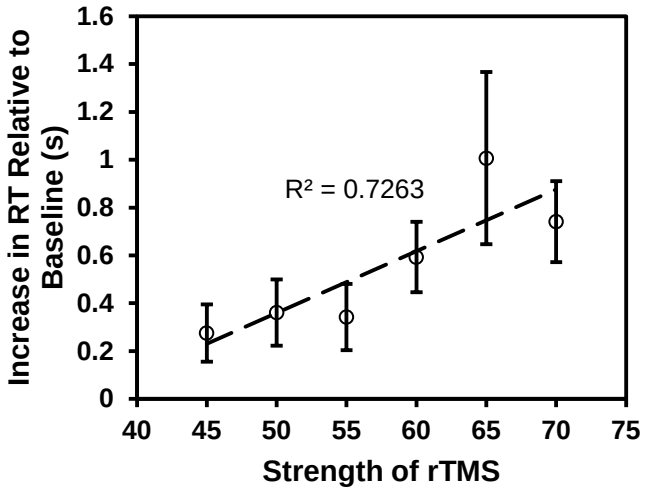

Figure 2.29. Scatter plot showing average response times (s) for the two pilot participants when TMS is applied to TO-1. Black dashed line denotes linear trendline. Error bars represent S.E.M.

2.12.5 Discussion

Firstly although these results comprise a small sample size (n=2), they have produced very reassuring findings suggesting that appropriate application of TMS to TO-1 will produce a deficit in performance for translational motion.

When TMS was applied to TO-1, one significant difference was identified between performance at 55% strength and baseline. Even during application of the weakest tested strength (45%) for the TO-1 conditions there was, on

average, a 7% reduction in the subjects' ability to perform accurate direction discriminations. This observed decrease in ability when TMS is applied to TO-1 is consistent with previous research showing that TMS applied to the hV5/MT+ complex affects performance on direction discrimination tasks (Beckers and Zeki, 1995). In contrast to this, when TMS was applied to LO-1, no significant difference was found. This is useful because it bolsters the premise that TMS itself should not disrupt performance, unless the targeted site is a motion-sensitive area. As LO-1 has not been shown to process moving stimuli (Larsson and Heeger, 2006), a lack of effect is a positive result.

The correlational analyses suggest that the strength of the TMS is directly related to the extent of the detrimental effect on performance ($r = -0.96$). This means the final strength of TMS chosen for the following experiments will be an important consideration in order to find measurable effects and ensure a sufficient experimental power.

In summary, it seems the most efficient method for testing the effects of TMS will be to use the highest strength tested: 70%. Although this did not produce a significant effect, it produced a larger effect than the 55% strength that did produce a significant reduction. It is concluded therefore that this is most likely to produce the biggest effect when TMS is applied to the target areas and therefore will give the experimental paradigm the best chance of finding functional differences between TO-1 and TO-2.

2.13 General Discussion of Methods

Overall, these data have identified that; 1) individual thresholds vary between subjects and across tasks, 2) dot density does not affect baseline thresholds, and 3) effects of TMS increase linearly with increasing strength. Taken together, this combination of results highlight that experiments will need to ensure to measure individual threshold levels before subjects participate in any experiments, and that relatively high strength of TMS will be necessary in order to produce measurable effects on performance.

Chapter 3
Application of Distal TMS to Areas TO-1 and TO-2

3.1 Overview

The proposed sub-divisions of hV5/MT+ have been located functionally in fMRI imaging studies several times over recent years (Dukelow et al., 2001; Huk et al., 2002; Amano et al., 2009; Kolster et al., 2010). There is also functional neuroimaging evidence to suggest that the anterior region (TO-2) will be more specialised for radial motion than translational (Smith et al., 2006). However, as fMRI analysis only implies a correlation between the type of stimuli and the region of interest, there is a need for causal evidence to allow conclusions of functional dissociations to be drawn.

Distal TMS is the application of low frequency TMS for a duration of time prior to the experiment (Walsh and Cowey, 2000; Kosslyn et al., 1999). It is thought that this type of stimulation works by reducing regional cerebral blood flow thereby producing reductions in ability to perform on behavioural tasks that require function of the targeted area. This provides direct, causal evidence for functionality of areas within the visual brain.

3.2 Hypothesis and Aims

This experiment aimed to be the first of a series with the intention of providing causal evidence for functional distinctions between areas TO-1 and TO-2 using fMRI-guided distal TMS. It utilised conclusions drawn from experiments involving both monkeys and humans in order to predict that the posterior division, TO-1, should be functionally responsible for processing

translational motion, while the anterior division, TO-2, is most likely to have a role in processing radial and rotational motion (Saito et al., 1986; Duffy and Wurtz, 1995; Orban et al., 1995; Smith et al., 2006; Wall et al., 2008). From this, it can be hypothesised that application of low-frequency, distal (or 'offline') TMS to TO-1 should reduce subjects ability to perform on the translational direction task, whilst application of TMS to TO-2 should diminish performance on radial and rotational tasks.

3.3 Methods

3.3.1 Subjects

Five subjects (mean age 29.6 years; range 21-46 years; two female) were recruited for this initial phase of experiments from an opportunity sample of students and staff at the University of Bradford and the University of York. Two of these subjects were naïve to the aims of the experiment (S4, S6). All subjects had normal or corrected to normal vision at the time of testing and had no history of neurological or psychiatric disorders. Subjects were fully informed of any possible health risks associated with fMRI and TMS procedures, and were required to read an information sheet outlining the exact protocol of the experiment and how to participate in the experiments safely. The subjects also filled in a questionnaire confirming that there were no contraindicating health risks for the procedure, and gave their full informed consent (see Appendices 1-3.

Experiments were approved by both relevant ethics committees (University of Bradford and York Neuroimaging Centre), and were conducted in

accordance with both the Declaration of Helsinki and accepted TMS safety protocols (Wassermann, 1998).

3.3.2 Identification of Target Sites

For this experiment, two experimental target sites (TO-1, TO-2) and one control site (LO-1) were chosen as regions of interest. These sites were identified in individual subjects as outlined in Chapter 2 (Section 2.6; Table 2.2), so target points were consistent within subjects across all three direction tasks.

3.3.3 Psychophysical Stimuli and Procedure

The motion paradigm tested in these experiments was direction discrimination via motion coherence. During these tests a low, but identifiable, percentage of dots will be moving in a coherent direction (signal dots) whilst the remaining dots translate randomly (noise dots). During testing, subjects identified the coherent direction of signal dots using a two-alternative-forced choice (2AFC) paradigm for three motion conditions: translational (up/down), radial (inward/outward), and rotational coherent dots (clockwise/anti-clockwise). Subjects recorded their decision regarding the direction of signal dots using an appropriate button on the keyboard.

The stimuli comprised a 10° circular aperture containing 300 white dots presented on a black background. Each dot subtended ~0.2° of visual angle (dot density ~4.69/deg^2) and regardless of direction, all dots moved at a speed of 7°/s as this has been suggested to fall within a range of velocities

that produce optimal activation in human motion-sensitive visual areas (Chawla et al., 1998; Becker et al., 2008). This aperture was horizontally displaced 10° to the left of the fixation point (see Figure 3.1). This positioning was utilised for two reasons; 1) it limits the contribution of motion-sensitive areas within the left hemisphere because it is positioned solely in the left visual field, and 2) it is far enough into the periphery that it should be possible to functionally distinguish between TO-1 and TO-2 based on their receptive field sizes. All dots were assigned a lifetime of 20 frames because this prevents subjects from ‘cheating’ as they were unable to follow the path of the dots with their gaze. On each trial, the percentage of coherently moving dots would comprise one of a range of seven levels tailored to the performance ability of the individual subject, and each percentage would be presented 25 times per run. For each average measurement of performance, the subjects were required to complete two runs of each direction per condition.

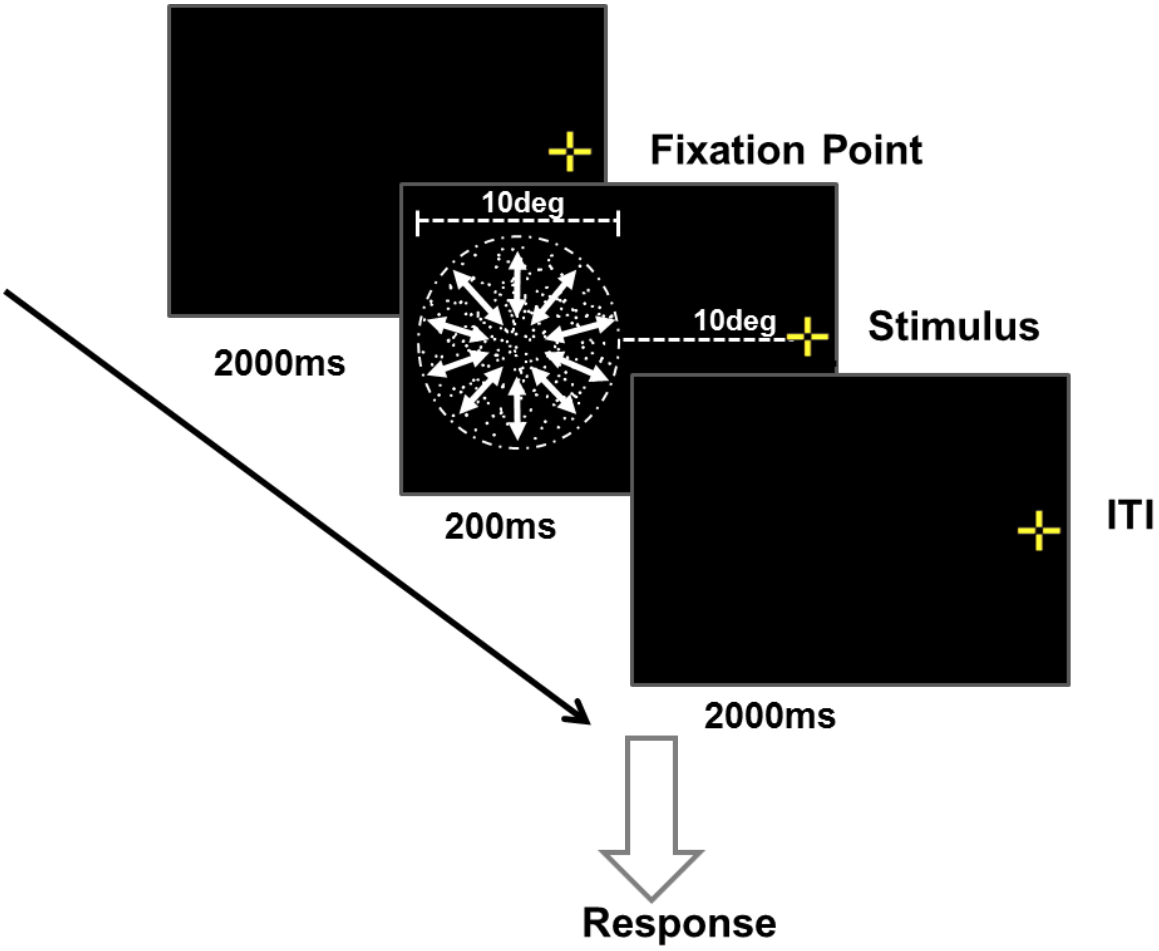

Figure 3.1. Schematic of psychophysical procedure using radial dots as an example stimulus. There is a 2000ms ITI, in which the participant is required to make a response. The stimulus is shown for 200ms.

Subjects viewed a centrally placed fixation cross with their right eye (left eye occluded) from a distance of 57cm (see Figure 2.14).

Once the full range of data was collected for each condition, psychometric functions were applied using the formula outlined previously (Chapter 2; Section 2.10.2.3; Eq. 3). Thresholds were then extracted as the 75% performance along the psychometric curve.

3.3.4 Distal TMS Protocol

For protocols using distal application of TMS, a low frequency of TMS is applied to the subject's scalp for a duration prior to the experimental task being performed. The TMS pulses were applied at a frequency of 1 Hz, at a level of 50% of the maximum output for 10 minutes immediately prior to taking part in the experiment. This technique is proposed to reduce blood flow beneath the coil within the target area which can cause an associated decrease in ability to perform on behavioural tasks associated with the function of that area (Walsh and Cowey, 2000). This effect on behavioural performance is reported to last for a duration equating to 50-200% of the duration of the TMS (Walsh and Cowey, 2000; Matsuyoshi et al., 2007). For instance, in this experiment, a train of 600 biphasic (equal relative amplitude) TMS pulses separated by 1000ms were applied to the subject's scalp for 10 minutes (Figure 3.2). This experimental paradigm allowed the potential for 5-20 minutes for duration of effect of the TMS.

TMS pulses were applied using a figure-of-eight coil (50 mm diameter) connected to a Magstim Rapid^2 stimulator (Magstim, Wales, UK). The coil was secured in a mechanical clamp and its position relative to the target ROI was monitored in real-time throughout the delivery of the pulses, so that any shift in position relative to the subject's head could easily be rectified. Subjects undertook two blocks of the motion task for each TMS site and condition in separate sessions.

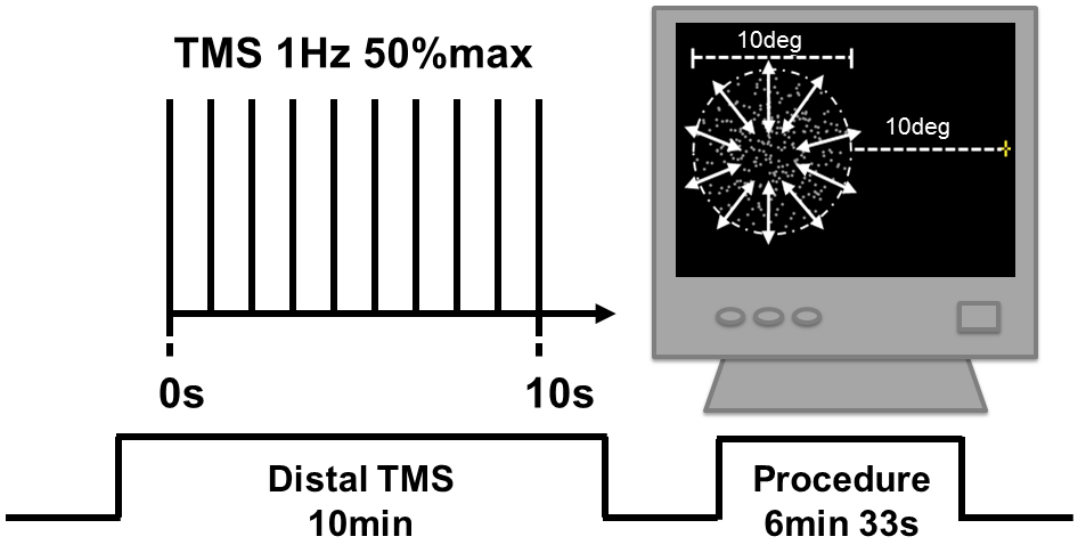

Figure 3.2. Timescale of distal TMS procedure outlining duration of TMS, and duration of psychophysics.

3.3.5 Data and Statistical Analysis

A small percentage of trials (~1%) were removed prior to statistical analysis if the TMS coil did not successfully deliver a pulse.

Statistical analyses were carried out using IBM SPSS Statistics 20, in which repeated measures ANOVAs were used to test threshold, response times, slope values and response biases for each task. The assumption of normal distribution was confirmed with Mauchly's Test of Sphericity. If this assumption was met (i.e. sphericity is non-significant) then the ANOVA was calculated assuming sphericity. However if the assumption was violated, the degrees of freedom (dF) were corrected to allow appropriate interpretation of the F value of the ANOVA. These dF corrections included the Greenhouse-Geisser when sphericity (ε) was reported as less than 0.75, and Huynh-Feldt correction when sphericity exceeded 0.75.

3.4 Results

3.4.1 Threshold

The average threshold values and response times for each condition are shown in Figure 3.3.

Whilst investigating differences in threshold value for the translational motion task, Mauchly's Test of Sphericity indicated that the assumption of sphericity had not been violated ($\chi^2(5) = 4.87, p = 0.458$) so no corrections were performed. The subsequent one-way repeated measures ANOVA highlighted no significant main effects of TMS condition on threshold values ($F(3,12) = 3.48, p = 0.050$).

Threshold values for radial motion did violate the assumption of sphericity ($\chi^2(5) = 13.65, p = 0.025$) so the epsilon value ($\varepsilon = 0.49$) determined which correction would be most appropriate. The Greenhouse-Geisser correction showed no significant main effect of applying TMS prior to the presentation of radial stimuli ($F(1.47,5.87) = 2.92, p = 0.136$).

For the rotational motion task, the assumption of sphericity was not violated ($\chi^2(5) = 5.10, p = 0.430$) so no corrections were applied. A one-way repeated measures ANOVA showed no significant differences across any of the TMS conditions for rotational motion ($F(3,12) = 2.15, p = 0.147$).

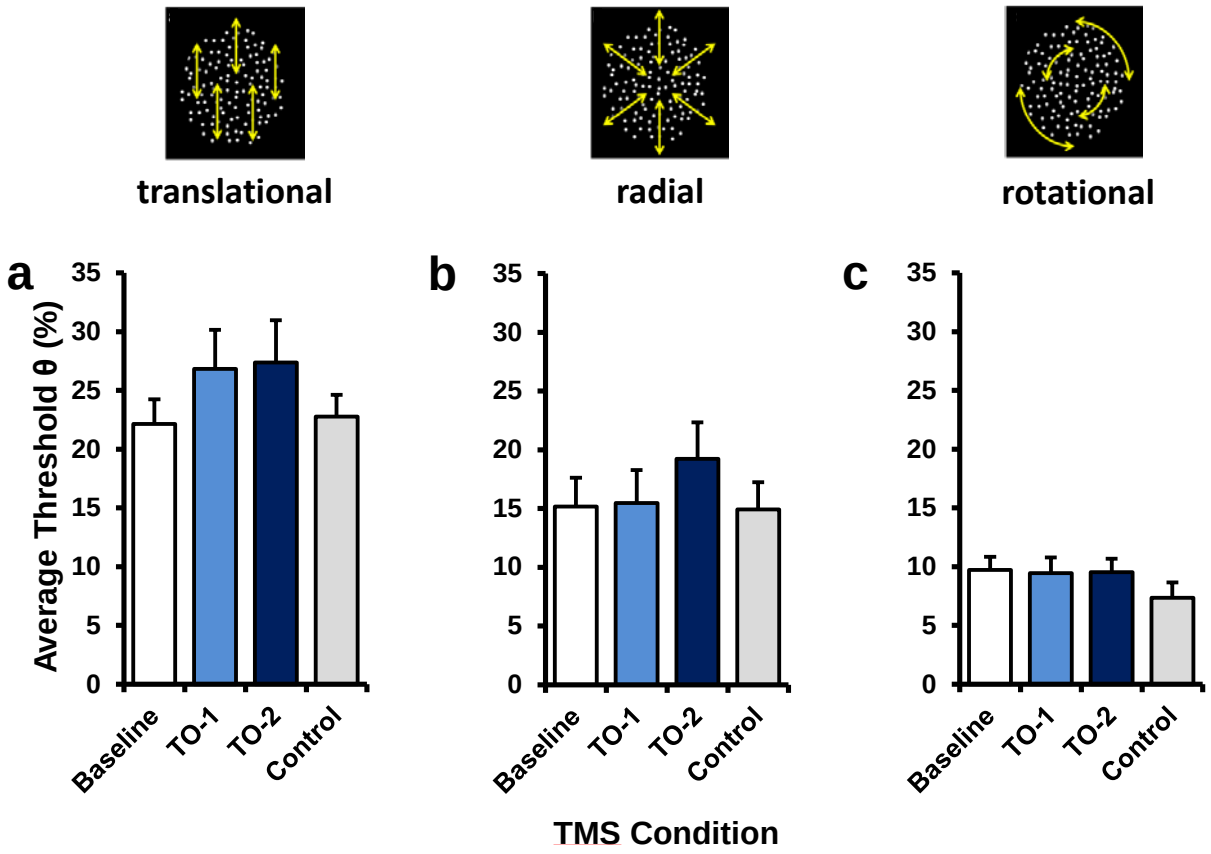

Figure 3.3. Bar charts showing average pCorrect for Translational (a), Radial (b), and Rotational (c) tasks (a high value suggests the task was found to be more difficult). Error bars show S.E.M.

3.4.2 Response Time

Response times were investigated for two reasons: 1) to ensure subjects were not taking too long to think about responses that should be at threshold, 2) to ensure that any effect is not due to the time it takes the subject to respond (Figure 3.4). Response time was measured (in milliseconds) as the amount of time taken to press a key following cessation of the stimulus.

For translational response time data (s), Mauchly's Test of Sphericity suggested that the assumption of sphericity had been violated ($\chi^2(5) = 11.81$, $p = 0.049$) so the epsilon value ($\varepsilon = 0.47$) determined the most appropriate

correction to use. The Greenhouse-Geisser correction showed response times do not differ with application of TMS within this motion direction ($F(1.41,5.65) = 1.83$, $p = 0.238$). Likewise for radial motion, although the assumption of sphericity was violated ($\chi^2(5) = 19.47$, $p = 0.003$; $\varepsilon = 0.36$), a Greenhouse-Geisser correction showed no significant main effect ($F(1.06,4.25) = 1.28$, $p = 0.322$). For rotational response times, Mauchly's Test of Sphericity suggested that the assumption of sphericity had not been violated ($\chi^2(5) = 3.58$, $p = 0.631$). A one-way repeated measures ANOVA showed response times did not significantly differ with application of distal TMS ($F(3,12) = 2.22$, $p = 0.139$).

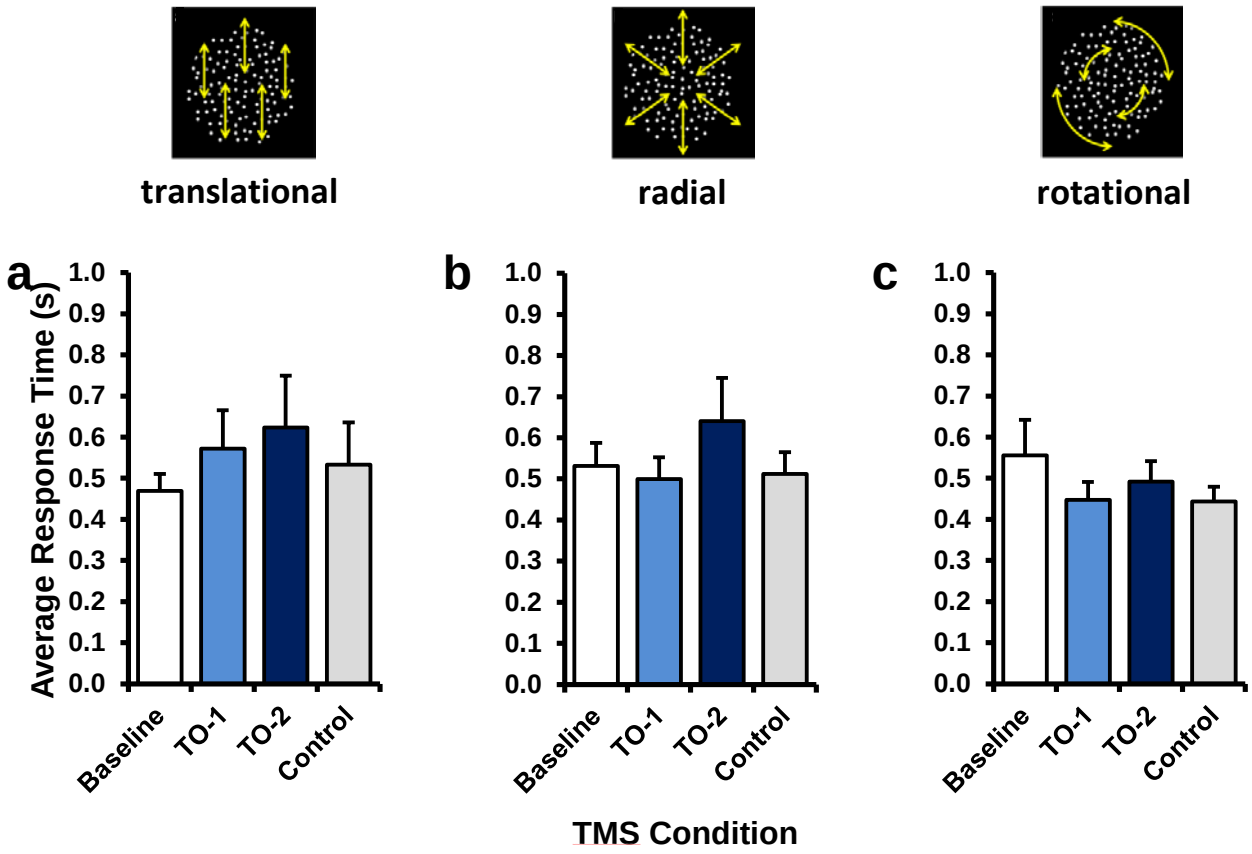

Figure 3.4. Bar charts showing average response time (s) data for Translational (a), Radial (b), and Rotational (c) tasks (a high value suggests a longer decision time). Error bars show S.E.M.

3.4.3 Slope Values

Analysis of the slope values was important for ascertaining whether the distal TMS affected subjects' sensitivity to the task. The slope value is extracted at the point of the threshold. A high value implies a steep slope (more sensitive to the task) whereas a low value implies a shallow slope (less sensitive to the task).

Repeated measures ANOVAs were performed across all three direction tasks in order to investigate the potential differences between TMS conditions (Figure 3.5).

A sphericity assumed ($\chi^2(5) = 3.10$, $p = 0.701$) repeated measures ANOVA on the translational data highlighted a significant main effect of TMS on slope sensitivity ($F(3,12) = 9.09$, $p = 0.002$), however subsequent bonferroni corrected pairwise comparisons did not identify any significant differences between conditions (Baseline *versus* TO-1, p = 0.183; Baseline *versus* TO-2, $p = 0.237$; TO-1 *versus* Control, $p = 0.102$; TO-2 *versus* Control, $p = 0.088$; for all other comparisons, $p = 1.00$).

For radial motion, sphericity was not assumed ($\chi^2(5) = 18.73$, $p = 0.004$) so the epsilon value was examined ($\varepsilon = 0.38$). The Greenhouse-Geisser correction showed no significant effect of applying TMS to slope sensitivity ($F(1.13,4.52) = 0.35$, $p = 0.607$).

Analysis of rotational motion (sphericity assumed; $\chi^2(5) = 1.99$, $p = 0.859$) showed no significant main effect on slope sensitivity ($F(3,12) = 0.65$, $p = 0.597$).

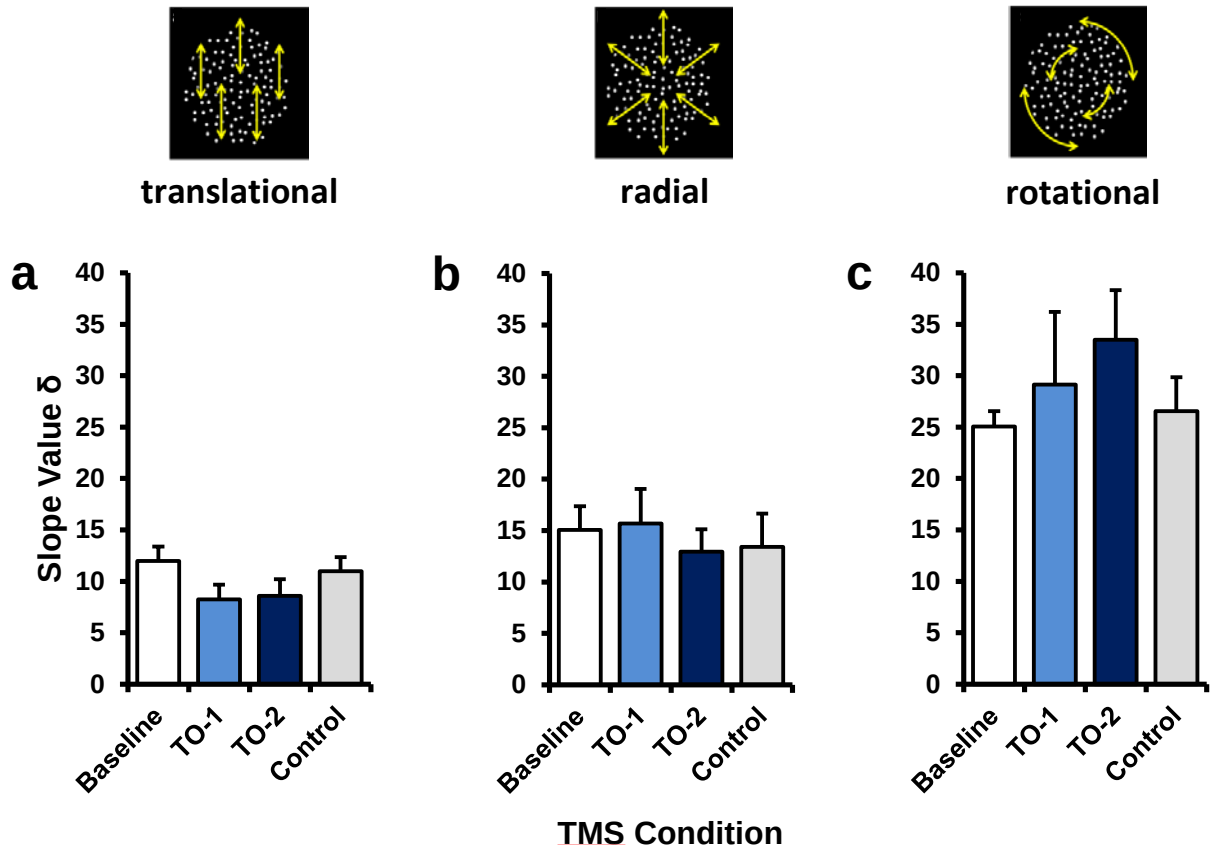

Figure 3.5. Bar charts showing average extracted slope values (at point of threshold on psychometric function) for Translational (a), Radial (b), and Rotational (c) tasks (a low value suggests the subject experienced a decrease in sensitivity to the task). Error bars show S.E.M.

3.4.4 Correlations between Threshold and Slope

Table 3.1. Table reporting means and standard deviations for threshold and slope values for the correlational analysis.

	Mean	Std. Deviation
Translational		
Threshold (% Coherent Dots)	24.78	6.26
Slope Value	9.95	3.38
Radial		
Threshold (% Coherent Dots)	16.21	5.79
Slope Value	14.28	5.90
Rotational		
Threshold (% Coherent Dots)	9.01	2.72
Slope Value	28.56	10.09

The slope values and the threshold values were investigated for correlations to determine whether an increase in threshold (performance) is likely to predict a decrease in slope (sensitivity) (Table 3.1; Figure 3.6). Pearson's R correlations highlighted significant negative correlations between slope and threshold values for translational and radial direction discrimination tasks (translational, r(20) = -0.80, $p < 0.001$; radial, r(20) = -0.52, $p = 0.019$. No significant correlation was found for rotational motion (r(20) = -0.41, $p = 0.076$).

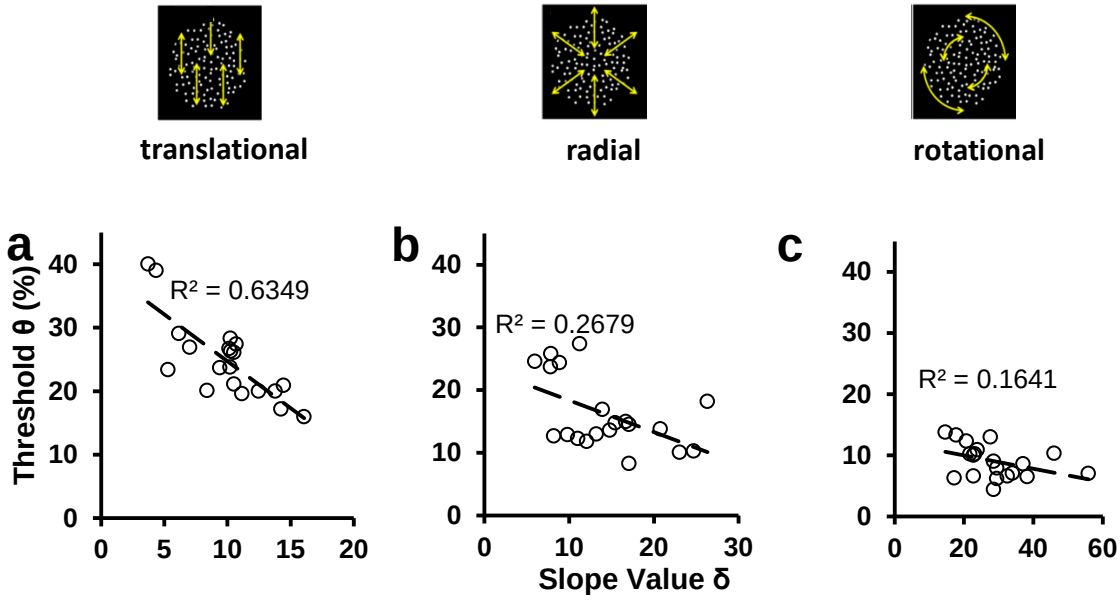

Figure 3.6. Scatter plots showing correlation between threshold (%) and slope values for each of the three tasks: translational (a), radial (b), and rotational (c). The dotted black lines denote linear trendlines for each condition and the R^2 is displayed on each plot.

3.4.5 Response Bias

Response bias values were calculated using the following formula (Eq. 8):

$$Bias = (Freq.of\ 'A' \div Total\ Trials) \times 100 - 50 \quad (8)$$

This formula converts the probability of A (given response) into a percentage. It then subtracts 50 in order to get the percentage of responses that were above or below chance (equivalent to 0).

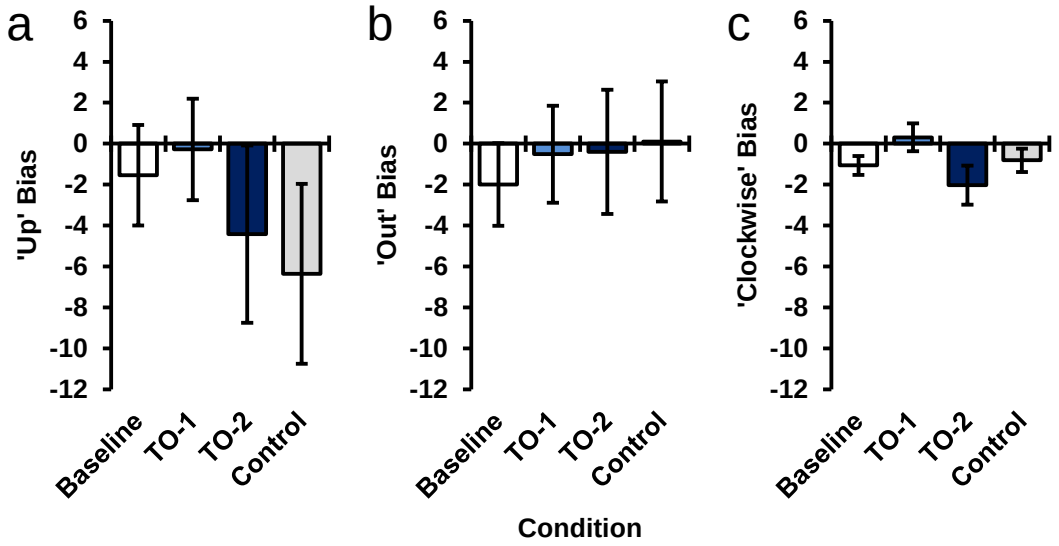

Figure 3.7. Bar charts showing response biases for each of the three tasks: translational (a), radial (b), and rotational (c). A negative bias would indicate the bias went in the opposite direction to the one described along the vertical axis. Error bars represent S.E.M.

Student t-tests were used in order to ascertain whether any of the average bias results were significantly different from 0, if found to be significant this would suggest a bias in a particular direction (Figure 3.7). No significant differences were found across any of the three direction tasks: translational (Baseline [t(4)= -0.63, p = 0.564]; TO-1 [t(4)= -0.11, p = 0.915]; TO-2 [t(4)= -1.02, p = 0.365]; Control [t(4)= -1.45, p = 0.221]), Radial (Baseline [t(4)= -0.99, p = 0.378]; TO-1 [t(4)= -0.22, p = 0.839]; TO-2 [t(4)= -0.13, p = 0.902]; Control [t(4)= 0.04, p = 0.973]), Rotational (Baseline [t(4)= -2.28, p = 0.085]; TO-1 [t(4)= 0.45, p = 0.673]; TO-2 [t(4)= -2.12, p = 0.101]; Control [t(4)= -1.41, p = 0.230]).

Following this, it was necessary to investigate whether any differences between conditions existed within each task. For translational motion, sphericity was assumed ($\chi^2(5) = 1.01$, $p = 0.964$) and a repeated measures ANOVA showed no significant difference between conditions ($F(3,12) = 1.66$, $p = 0.229$).

Statistical analysis of the radial task (sphericity assumed; $\chi^2(5) = 8.00$, $p = 0.179$) also showed no significant difference between conditions ($F(3,12) = 0.43$, $p = 0.738$).

A repeated measures ANOVA performed on the rotational motion data (sphericity assumed; $\chi^2(5) = 2.92$, $p = 0.728$) did not show any significant difference between conditions ($F(3,12) = 2.58$, $p = 0.102$).

3.5 Discussion

These results reveal no significant differences in function of the sub-divisions TO-1 and TO-2. No significant effects of TMS condition on performance were found for any of the three direction discrimination tasks: translational, radial or rotational. This means that despite observed trends in the data suggesting application of distal TMS to areas TO-1 and TO-2 prior to presentation of translational motion increases threshold, and likewise, application of distal TMS to TO-2 prior to radial motion increases average threshold; no conclusions can be drawn as to the validity of these trends as they are not significantly different to one another.

This lack of significant difference contradicts previous research both in non-human primates and neuroimaging studies in humans. It would be expected

that distinct motion-sensitive areas would have differing cognitive functions, and previous research has shown that, similar to non-human primates, anterior area TO-2 shows a greater increase in preferential activation for expanding stimuli relative to that observed within TO-1 (Smith et al., 2006). This would propose a functional difference should exist between TO-1 and TO-2, particularly between translational and radial motion, however the lack of evidence in this experiment would seem to argue against that.

Despite this, it could be argued that as there are trends in the data that imply a difference may exist, and given that any application of TMS to area hV5/MT+ as a whole would be expected to find a significant reduction in subjects' ability to perform on motion tasks (Matthews et al., 2001; McKeefry et al., 2008; McKeefry et al., 2010), that perhaps the lack of any behavioural deficit reported here may be more likely to be a consequence of the methodology used. For example, it is likely that the experimental power may not be particularly strong (P = 0.41) given the weak strength of TMS used and the limited number of subjects. Previous preliminary experiments also showed that during online (concurrent) application of TMS, higher pulse strengths correlate with larger behavioural disruptions (see Chapter 2; Section 2.12). If in future experiments, the experimental paradigm could be changed to an online TMS protocol (potentially at a higher power), this should create a larger difference in effect which may allow these trends to reach significance.

There is also a risk that due to the nature of the proximity of the two regions, no functional distinction will ever be successful using TMS as they are most often located within a sulcus (Dumoulin et al., 2000). This means that not

only are they likely to be extremely close together, but that they are also less likely to be superficial cortical regions of interest. This could provide a limitation as the proximity of TO-1 and TO-2 requires use of a small coil size, thereby reducing the spread of the magnetic field and reducing the probability of inadvertently stimulating any neighbouring regions. However this small coil size also reduces the depth of the field so it is possible that deeper cortical regions may be beyond the reach of the smaller coil (Tofts, 1990; Ren et al., 1995; Walsh and Cowey, 2000). The only way to discern whether this is a limitation that can feasibly be overcome will be to do further experiments using a stronger power of TMS in order to increase the potential for finding functional differences.

One particularly successful fragment of this experimental design has been that no significant difference was found between baseline and control performance across any of the three direction discrimination tasks. This reiterates the confidence in the decision to use visual area LO-1 as the control site because it is not a motion-sensitive area and applying TMS to this region does not induce a pseudo-reduction in subjects' ability. This asserts that TMS is a method that is reliable and fit for purpose.

The lack of significant difference across the response times for any of the conditions or tasks suggests that the subjects' response time cannot have contributed to the significant main effect found for slope sensitivity during the translational motion task. It was hypothesised that response times may be slower when cognitive demand is higher i.e. if the task is more difficult, response times may increase. However this has not been found to be the case. It could be argued that this is likely due to the lack of differences in

performance found across the various conditions. This means if the experimental methodology is improved, it will be important to analyse the response times again and see if the perceived difficulty of the task has an impact on speed of response.

Analysis of the slope values extracted from the psychometric function produced a significant main effect across conditions during the translational motion task, however pairwise comparisons failed to identify a significant difference between TMS conditions. This makes it difficult to make specific conclusions regarding any effects at this stage. No significant effects were found for radial or rotational tasks, suggesting the average values were not significantly different between conditions. This slope value is a measure of sensitivity to the task, so although it can be concluded that sensitivity has not been affected by the application of TMS to either of the experimental sites (TO-1 or TO-2), this is evidence that an improved method is required in order to determine whether the significant main effect across translational motion is a genuine product of the application of TMS or not.

The correlational analysis performed between threshold and slope values revealed significant negative correlations on the translational and radial tasks. This means that for those two directions, a decrease in performance (high threshold) is related to a decrease in task-related sensitivity (low slope value). This demonstrates that in this instance, both bias (threshold) and sensitivity are associated. No significant correlation was reported for the rotational task, but this could be due to the narrow comparative range of threshold values acquired for rotational motion (4.4%-13%). This was highlighted as a potential issue in Chapter 2.10 and so in future experiments,

it would be beneficial to have a dependent variable that will remain consistent across tasks (e.g. percent correct) in order to be better equipped to compare performances.

The data obtained on response biases sought to determine whether subjects had been making appropriate decisions on the task. Any recorded data would have been invalid if a subject only guessed one direction when behaviourally impaired during TMS conditions. This analysis allowed a way of monitoring percentage of responses that were in a particular direction. In all cases, there was an even number of dots moving in each of the two possible directions. This meant that subjects should guess 50% for each direction. However, there are always natural biases and there is the possibility of errors, so it is unlikely to be exactly 50% for each individual. The results in Figure 3.7 show that responses tended to hover around a 0% bias with no significant differences across any of the tasks. This means that it is likely the subjects were responding appropriately on the task.

The overarching limitation of this experiment, however, is the combined low strength (50%) and frequency (1Hz) of the distal TMS method that was chosen due to the 50mm coils being more likely to overheat very quickly at higher strengths and frequencies (Walsh and Cowey, 2000). The proposed idea then is to use a different type of TMS such as an online TMS method in which repetitive TMS is concurrent with presentation of the stimuli as opposed to being presented in a block prior to the task. This will allow much shorter experimental sessions and consequently allow a higher strength of TMS to be used. This may increase the observed trends for performance high enough to become significant, and as a result it will be possible to

determine whether there are functional differences between TO-1 and TO-2 in human motion-sensitive cortex.

To summarise, although this experiment has not produced evidence to suggest that hV5/MT+ contains two functionally distinct visual areas (TO-1 and TO-2), however it has proven useful in terms of highlighting potential methodological improvements. This means the next step will be to repeat the experiment using better developed TMS protocols as outlined in the previous Chapter (Chapter 2; Section 2.12). This will involve higher frequency and higher strength TMS presented concurrently with (as opposed to prior to) the task.

Chapter 4.1
Functionally Distinguishing Between TO-1 and TO-2 Using Repetitive TMS

4.1.1 Introduction

The main aim of this experiment was to investigate whether two sub-divisions of hV5/MT+ (TO-1 and TO-2) have distinct and measurable functional properties. Previous experiments (Saito et al., 1986; Duffy and Wurtz, 1991a) have shown that these areas in non-human primates are functionally dissociable. The posterior area MT (thought to correspond to TO-1) is involved in the analysis of translational motion, whilst the anterior area MST (thought to correspond to TO-2) has been shown to be more involved in the analysis of complex radial and rotational motion stimuli (Saito et al., 1986; Duffy and Wurtz, 1991a; Duffy and Wurtz, 1991b). Recent human neuroimaging experiments have shown these areas produce increases in activation when viewing these types of stimuli, but the literature has yet to causally establish their specific functional contributions to the perception of these different kinds of motion stimuli (Smith et al., 2006; Wall et al., 2008).

Using the same psychophysical procedure as the previous distal TMS experiment (see Chapter 3) involving direction discrimination on translational, radial and rotational motion tasks, this experiment aimed to use TMS to establish causal evidence for functional differences between these hV5/MT+ sub-divisions (TO-1 and TO-2). However, in contrast to the previous experiment, this one utilised an online (concurrent) TMS paradigm (Walsh and Cowey, 2000). It was hypothesised that this technique should produce

stronger detrimental effects on performance and therefore produce measurable results (as indicated in preliminary experiments investigating effects of strength of TMS; see Chapter 2; Section 2.12).

4.1.2 Hypothesis and Aims

Based on evidence from previous human neuroimaging experiments (Smith et al., 2006; Wall et al., 2008), it was hypothesised that application of TMS to TO-1 would produce a reduction in ability for the translational motion task, whilst TMS delivered to TO-2 would affect subjects' ability to perform appropriately on the radial and rotational tasks. The application of TMS to the control site (LO-1) should produce no measurable effects on the motion tasks.

4.1.3 Methods

4.1.3.1 Subjects

Six subjects from the original seven (mean age 30.5 years; range 21-46 years; two female) were recruited from the University of Bradford. Three of these subjects (one female) were naïve to the aims of the experiment (S4, S6, S7) and all experimental procedures were single-blind. All subjects had normal or corrected-to-normal vision at the time of testing and had no history of neurological or psychiatric disorders. Subjects were fully informed of any possible risks associated with fMRI and TMS procedures, and were required to read an information sheet outlining the exact protocol of the experiment

and how to participate in the experiments safely. Subjects signed a health questionnaire before every testing session.

Experiments were approved by ethics committees at both the University of Bradford and York Neuroimaging Centre, and were carried out in accordance with the Declaration of Helsinki and accepted TMS safety protocols (Wassermann, 1998; Lorberbaum and Wassermann, 2000).

4.1.3.2 Identification of Target Sites

All TMS target site co-ordinates remained the same as those outlined in Chapter 2 (Figure 2.10; Table 2.2). Experimental sites included TO-1, and TO-2, whilst the control site was LO-1.

4.1.3.3 Psychophysical Stimuli and Procedure

The three tasks tested were identical to that described in Chapter 3 in terms of dot characteristics and types of motion (translational, radial, and rotational). However, instead of testing points across a range of difficulties, this experiment used individual subjects' 75% thresholds in order to measure percentage correct (pCorrect). This was implemented as a response to the issues that arose in experiments described in Chapter 2.10 when comparing thresholds across the three tasks. It was clear that performance and ability varied between subjects and across tasks (i.e. the rotational task was perceived to be easier than the translational task). This meant thresholds varied from an average of 24.2% for translational to an incredibly low 8.7% for rotational (see Chapter 2; Table 2.3).

A solution to this was to create individual MATLAB codes that only used the threshold level of motion coherence for each condition. This meant that performance should stay relatively consistent between subjects and across tasks unless affected by the experimental procedure.

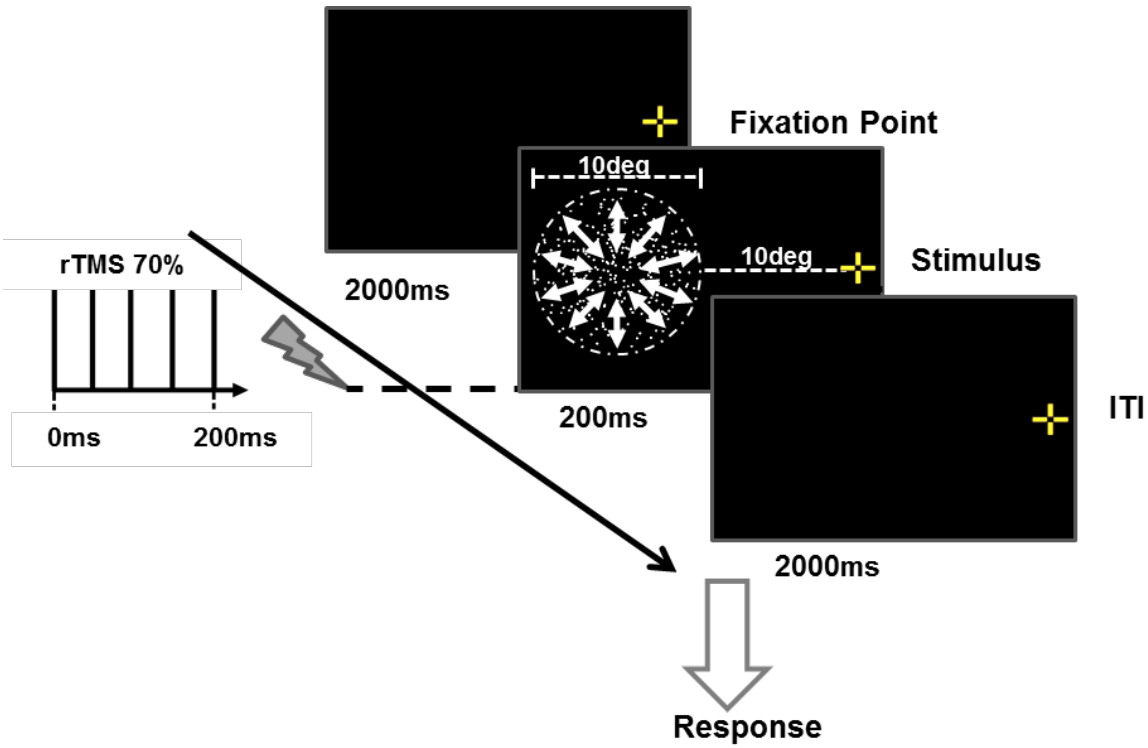

Figure 4.1. A schematic of combined psychophysical/TMS procedures using radial motion as an example task. There is a 2000ms ITI, in which the subject is required to make a response. The stimulus is shown for 200ms and onset of TMS and stimuli are synchronous. This procedure is used for three direction types: translational, radial and rotational.

Subjects saw 50 presentations of each stimulus per run and were required to sit for two runs per condition for each direction (total 100 trials per condition). The order of presentation of conditions was counter-balanced across subjects (Figure 4.1).

4.1.3.4 TMS Protocol

Repetitive biphasic trains of TMS were delivered concurrently with the onset of the stimulus and endured for an equivalent duration of 200ms. They were applied at 70% strength (as appropriately identified in Chapter 2.12), at a frequency of 25Hz for a duration of 200ms. This meant each train comprised of 5 pulses (See Figure 4.1). Accuracy of the registration of the coil was monitored in real time using a TMS Neuronavigation package for the BrainVoyager QX software. If the coil's position deviated by more than 2mm away from the target point during a trial, the trial was excluded from the analysis.

4.1.3.5 Data and Statistical Analysis

Statistical analyses were carried out using IBM SPSS Statistics 20, in which repeated measures ANOVAs were used to test percentage correct, response times and response biases for each task. Repeated-measures ANOVAs were calculated across all conditions (baseline, TO-1, TO-2, control). If Mauchly's test of sphericity reported a significant value then an appropriate correction was made. Any epsilon (ε) value below 0.75 corrected the degrees of freedom using a Greenhouse-Geisser correction, whereas an epsilon value above 0.75 required the use of a Huynh-Feldt correction. Significant main effects were investigated using two-tailed pairwise comparisons (Bonferroni corrected for multiple comparisons).

Some trials (~3%) were removed prior to statistical analysis because the TMS coil did not deliver a pulse for those particular trials and so any data would have been invalid.

4.1.4 Results

4.1.4.1 Percent Correct

Percent correct was the main dependent variable measured within this experiment. This variable quantifies the variance in performance around a 75% threshold as a function of both the task performed and TMS stimulation condition. Figure 4.2 shows that application of TMS to TO-1 and TO-2 appears to produce task-specific effects.

For translational direction discrimination, (sphericity assumed; $\chi^2(5) = 9.08$, $p = 0.117$) a repeated-measures ANOVA highlighted a significant main effect of application of TMS on performance ($F(3,15) = 20.17$, $p < 0.001$). Further pair-wise comparisons indicate that this effect is due to significant differences existing between the following conditions: Baseline and TO-1 ($p = 0.040$), Baseline and TO-2 ($p = 0.012$), Control and TO-1 ($p = 0.036$), and Control and TO-2 ($p = 0.019$). All other comparisons failed to demonstrate a significant difference ($p = 1.00$).

The radial direction discrimination task, (sphericity assumed; $\chi^2(5) = 2.94$, $p = 0.717$) also exhibited a significant effect of TMS condition on percent correct ($F(3,15) = 9.63$, $p = 0.001$). Pair-wise comparisons for this task indicated that this effect is due to significant differences existing between Baseline and TO-2 ($p = 0.027$), and Control and TO-2 ($p = 0.024$). All other comparisons failed

to demonstrate any significant differences (TO-1 *versus* TO-2, $p = 0.055$; all other comparisons, $p = 1.00$).

Rotational direction discrimination (sphericity assumed; $\chi^2(5) = 4.87$, $p = 0.446$) produced a significant main effect of TMS condition on performance ($F(3,15) = 4.27$, $p = 0.023$). However subsequent pair-wise comparisons indicated that there are no significant differences existing between individual conditions for rotational motion (Baseline *versus* TO-1, $p = 0.616$; Baseline *versus* TO-2, $p = 0.185$; Baseline *versus* Control, $p = 0.257$; TO-2 *versus* Control, $p = 0.378$; all other comparisons, $p = 1.00$). These results indicate that both TO-1 and TO-2 must be necessary for processing translational motion, whereas only area TO-2 is necessary for radial motion.

A two-way ANOVA was conducted to analyse effect of task and TMS condition on performance. Results reveal no significant interaction between motion direction and TMS condition ($F(6,60) = 1.71$, $p = 0.134$).

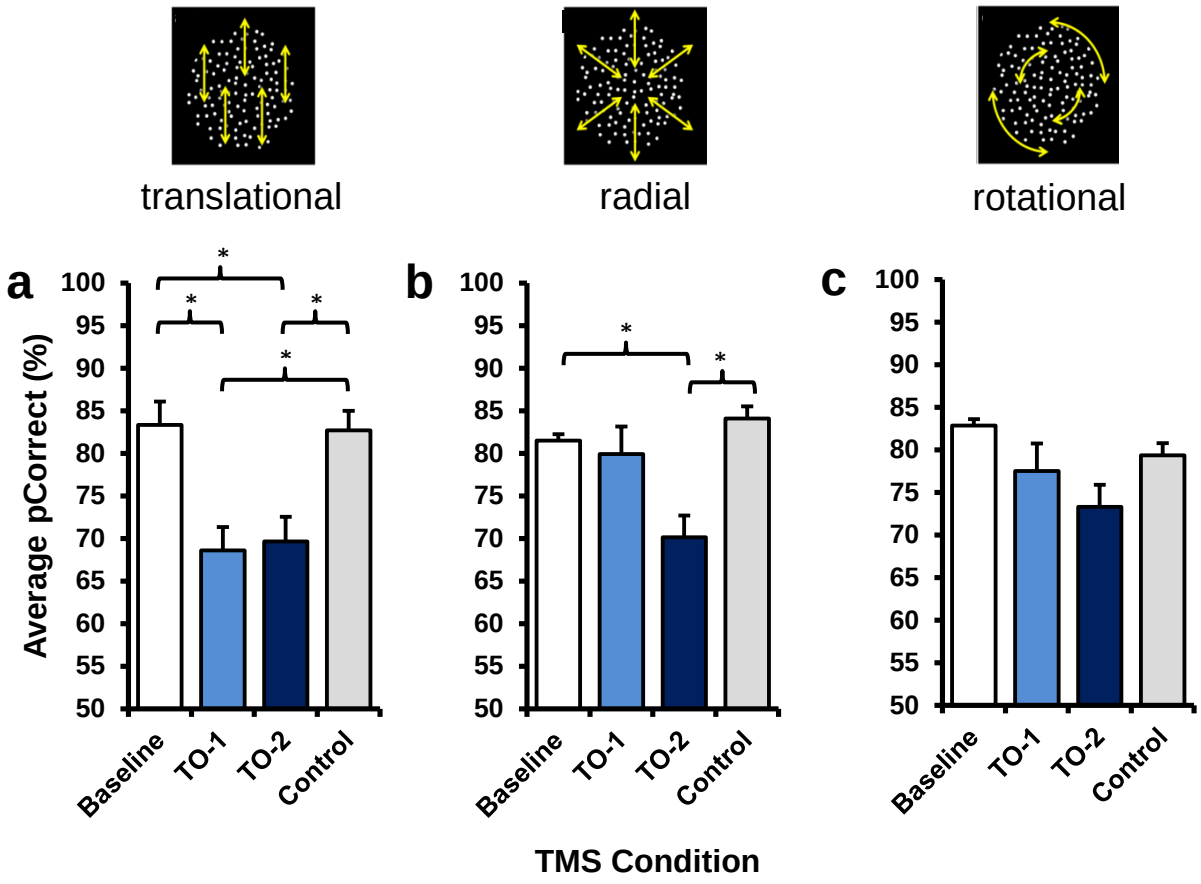

Figure 4.2. Bar charts showing average percent correct (pCorrect) across the three tasks: translational (a), radial (b), and rotational (c). Asterisks represent significance at the *p*=0.05 level. Error bars represent S.E.M.

4.1.4.2 Response Times

Analysis of response time (s) data collected for the three tasks (Figure 4.3) did not highlight any significant effects across translational (sphericity not assumed; $\chi^2(5) = 13.43$, $p = 0.024$; $\varepsilon = 0.441$; $F(1.32,6.62) = 3.71$, $p = 0.093$), radial (sphericity assumed; $\chi^2(5) = 9.63$, $p = 0.096$; $F(3,15) = 2.16$, $p = 0.135$) or rotational motion (sphericity assumed; $\chi^2(5) = 5.89$, $p = 0.332$; $F(3,15) = 0.76$, $p = 0.532$). This shows that time taken to respond probably did not have any effect on the observed differences for percent correct.

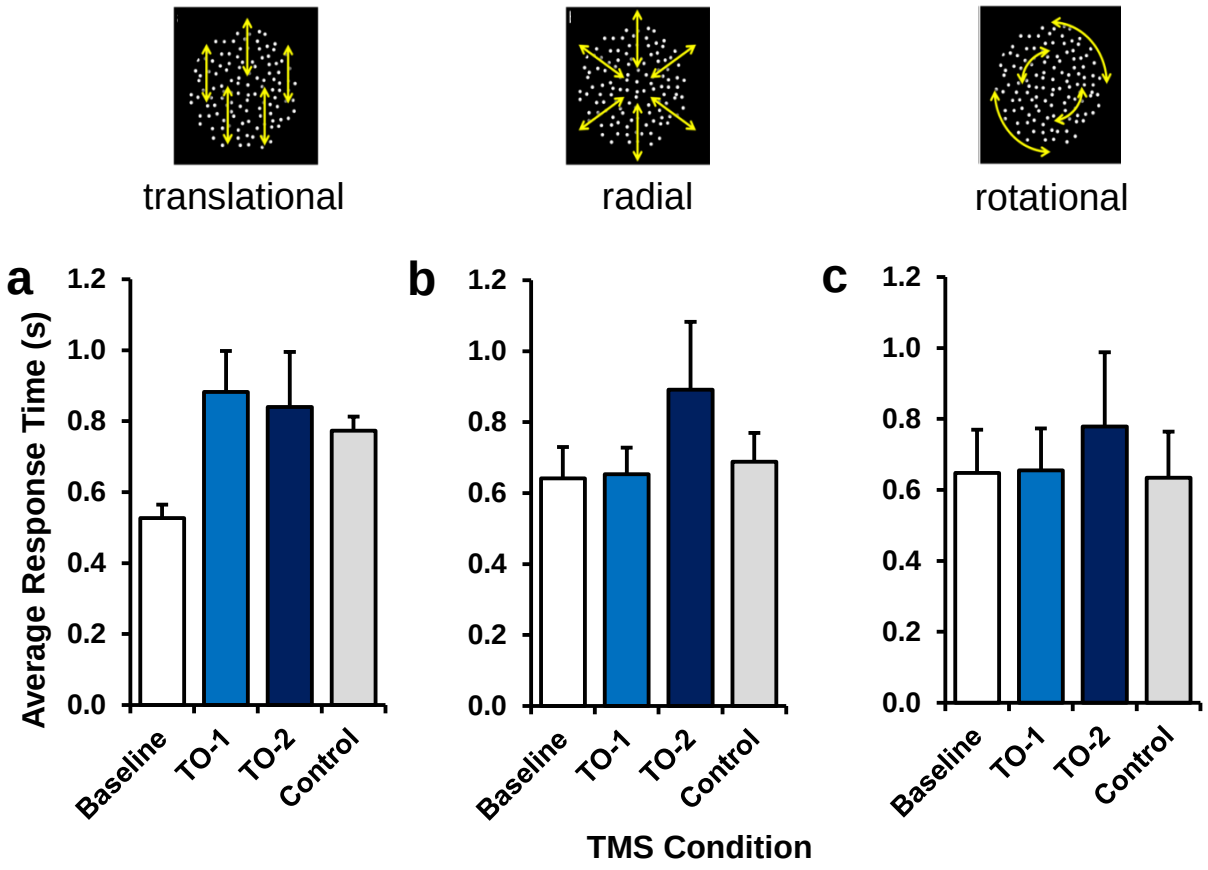

Figure 4.3. Bar charts showing average response times (s) across the three tasks: translational (a), radial (b), and rotational (c). Error bars represent S.E.M.

4.1.4.3 Response Biases

Response bias values were calculated as described in Chapter 3 (Section 3.4.5; Equation 8). The first stage of statistical analysis involved running student t-tests in order to ascertain whether any of these average bias results were significantly different from 0 (Figure 4.4). A value significantly removed from the 0 mark would suggest that subjects preferred a particular direction. No significant differences were found across any of the three direction tasks: translational (Baseline [t(5)= 0.17, p = 0.873]; TO-1 [t(5)= -0.26, p = 0.806]; TO-2 [t(5)= 0.53, p = 0.620]; Control [t(5)= 0.02, p = 0.988]),

Radial (Baseline [t(5)= 0.08, p = 0.943]; TO-1 [t(5)= -0.97, p = 0.377]; TO-2 [t(5)= 0.81, p = 0.457]; Control [t(5)= -1.05, p = 0.342]), Rotational (Baseline [t(5)= -0.16, p = 0.883]; TO-1 [t(5)= -0.78, p = 0.470]; TO-2 [t(5)= -0.91, p = 0.403]; Control [t(5)= -0.19, p = 0.855]).

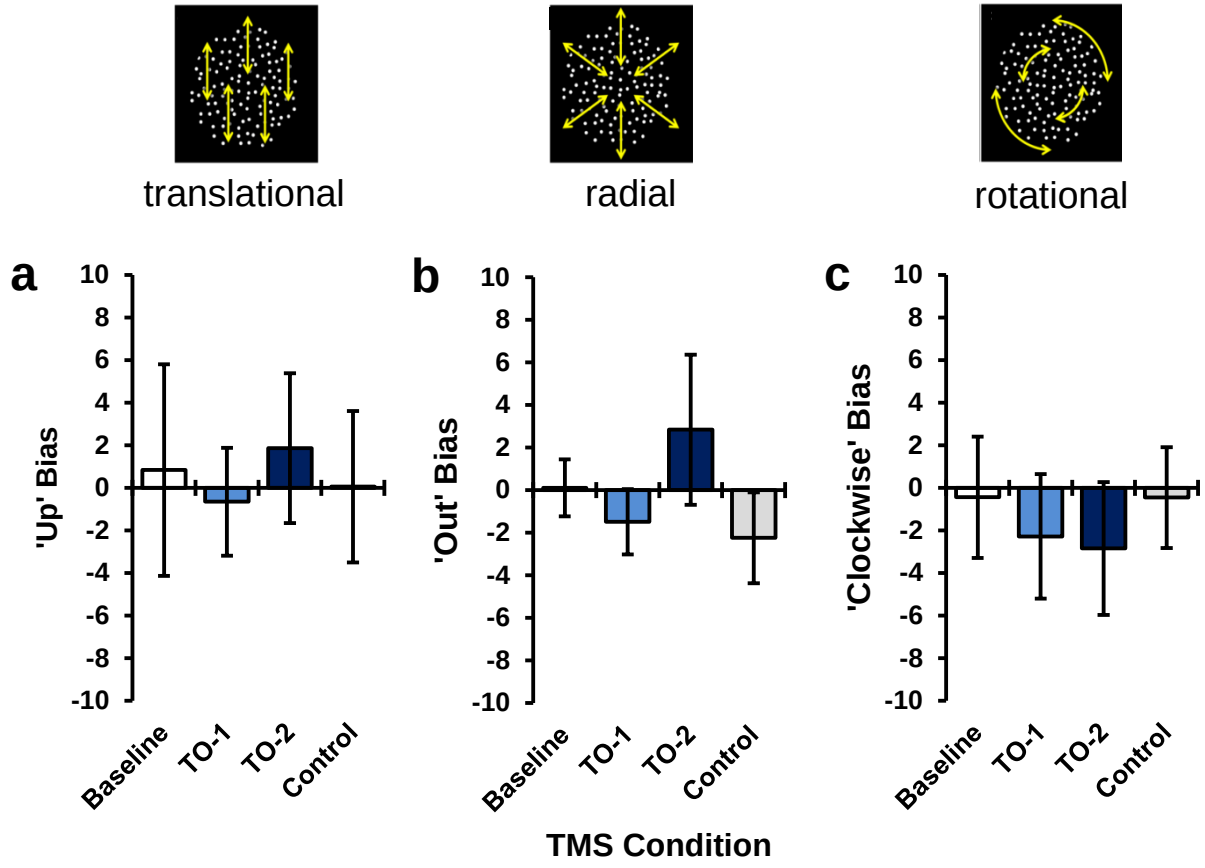

Figure 4.4. Bar charts showing response biases for each of the three tasks: translational (a), radial (b), and rotational (c). A negative bias would indicate the bias went in the opposite direction to the one described along the vertical axis. Error bars represent S.E.M.

The second stage of statistical analysis sought to investigate whether there were any differences between conditions within each task using repeated-measures ANOVAs. For translational motion, sphericity was not assumed ($\chi^2(5)$ = 13.78, p = 0.021) and a corrected Greenhouse-Geisser (ε = 0.47)

analysis demonstrated no significant difference between conditions ($F(1.42,7.08) = 0.21$, $p = 0.746$). Statistical analysis of the radial task (sphericity assumed; $\chi^2(5) = 5.30$, $p = 0.394$) also showed no significant difference between conditions ($F(3,15) = 2.26$, $p = 0.123$). A repeated measures ANOVA performed on the rotational motion bias data (sphericity assumed; $\chi^2(5) = 4.92$, $p = 0.440$) did not show any significant difference between conditions ($F(3,15) = 0.42$), $p = 0.741$).

4.1.4.4 Speed-Accuracy Analysis

In order to determine whether there was any effect of a speed-accuracy trade-off (i.e. subjects responding quickly at the cost of performing accurately), bivariate Pearson's R correlations were performed for each task (translational, radial, rotational).

Table 4.1. Table reporting means and standard deviations for percent correct and response times for the correlational analysis.

	Mean	Std. Deviation
Translational		
Percent Correct (%)	76.08	9.36
Response Times (s)	0.76	0.27
Radial		
Percent Correct (%)	78.92	7.40
Response Times (s)	0.72	0.29
Rotational		
Percent Correct (%)	78.26	7.97
Response Times (s)	0.68	0.35

Both translational ($r = -0.42$, $n = 24$, $p = 0.041$) and radial ($r = -0.43$, $n = 24$, $p = 0.034$) tasks were found to have significant moderately negative correlations between response time and percent correct. However, the

rotational task did not produce a significant correlation (r = 0.21, n = 24, *p* = 0.326). This can be interpreted as showing that a slower response in either the translational or radial motion tasks is correlated with a weaker performance on the task (Table 4.1; Figure 4.5). This is the opposite of a speed-accuracy trade-off.

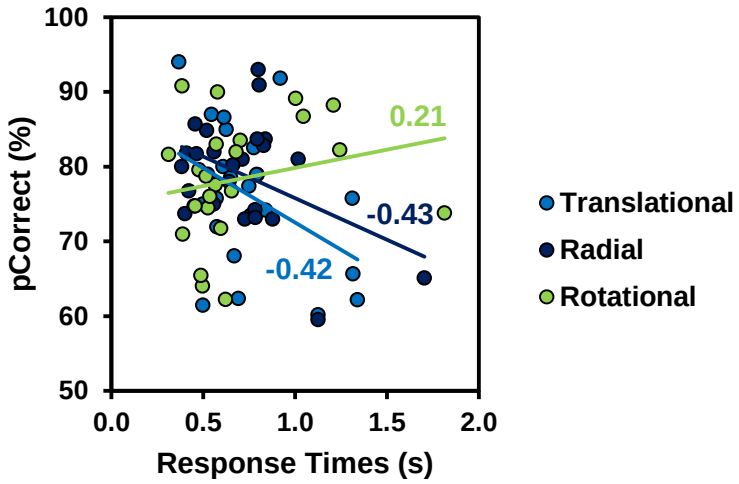

Figure 4.5. Scatter plot showing relationship between percent correct (%) and response time (s) for each of the three tasks (translational, radial, rotational). Colour-coded trendlines denote linear relationship, r values are shown on plot.

4.1.5 Discussion

These results show that areas TO-1 and TO-2 appear to be necessary for processing translational and radial motion. It is clear that application of TMS to TO-1 disrupts normal perception of translational motion, whilst application of TMS to TO-2 disrupts perception of both translational and radial motion. Previous neuroimaging experiments have successfully distinguished between these sub-divisions of hV5/MT+ based on RF properties and retinotopic representation (Dukelow et al., 2001; Huk et al., 2002; Amano et

al., 2009; Kolster et al., 2010), and have also provided correlative evidence suggestive of functional differences between TO-1 and TO-2 (Smith et al., 2006; Wall et al., 2008; Pitzalis et al., 2013c). However, this study has provided causal evidence of functional differences between TO-1 and TO-2 during processing of radially moving stimuli.

These results are largely consistent with findings from single-cell recordings of similar areas within non-human primates (Saito et al., 1986; Duffy, 1998). For example, area TO-1 appears to be restricted to processing translational motion which is a task closely associated with area MT in the monkey (Saito et al., 1986; Tanaka and Saito, 1989; Duffy and Wurtz, 1991a; Duffy and Wurtz, 1991b; Lagae et al., 1994). Similarly cells within MSTd are often associated with the perception of expanding/contracting stimuli (Saito et al., 1986), which corresponds to the proposed function of TO-2 in this study. However receptive field size of neurons within TO-2 appear to correlate more with that of MSTl/v (Amano et al., 2009), which is an area more closely associated with analysis of pursuit eye movements (Komatsu and Wurtz, 1989; Eifuku and Wurtz, 1998). This shows that although there is an element of homology between MT/ MST in the monkey and TO-1/ TO-2 in humans, it is still unclear as to whether TO-2 is the homologue to area MSTd, MSTl/v, or whether it is divergent from our current understanding of this complex.

The human neuroimaging literature asserts that neurons within TO-2 increase in activation and adapt to expanding stimuli, consistent with the idea that this area has been found to be responsible for processing expanding and contracting motion (Smith et al., 2006; Wall et al., 2008; Pitzalis et al., 2013c). However, Smith and colleagues (Smith et al., 2006; Wall et al., 2008)

also found evidence for TO-2 being involved in processing of rotational motion, which is not evident in this study. It seems therefore that these results are closer in line with those of Pitzalis et al. (2013c) who describe that neurons within TO-2 seem to be less responsive to rotating stimuli than translating or radiating stimuli.

This lack of significant effect for application of TMS during rotational motion tasks was unexpected, as it is known that cells within MST in non-human primates show an increase in firing rate when viewing rotational motion (Saito et al., 1986). However, further investigation within the literature highlights that relative to those present for radial motion, there are relatively less direction-selective cells sensitive to rotational motion within MST (Saito et al., 1986). Also, case study evidence of a patient with damage to right hV5/MT+ (encompassing both TO-1 and TO-2) has shown that although this patient's ability to perceive radial motion had diminished, her ability to perceive rotational motion was preserved (Beardsley and Vaina, 2005a; Beardsley and Vaina, 2005b). This is in agreement with the results presented here as a 'temporary lesion' induced by application of TMS produced the same pattern of impairment. This suggests that although neuroimaging evidence shows TO-1 and TO-2 are involved in the processing of rotating motion (Smith et al., 2006), accurate and successful perception of rotational direction must rely on function of an area external to this hV5/MT+ complex.

It is important to note that this experiment has only revealed a single dissociation between TO-1 and TO-2 as it is only the radial task which exposes a functional difference between the two areas. This suggests that TO-1 and TO-2 may be processing these tasks serially as opposed to

independently. In theory, this correlates with what is understood of non-human primates, as areas MT (the ‘general purpose motion processor’) transmits signals to higher areas for more complex analysis (Newsome and Paré, 1988). This infers a serial (as opposed to parallel) processing pathway. Also, previous experiments have shown that sensitivity to optic flow seems to result from a summation of signals across receptive fields of MST neurons that are locally selective to translational motion (Yu et al., 2010). If this is the case in the human brain, and area TO-2 is processing local translational motion in order to process global radial motion, it would be expected that application of TMS to TO-2 should disrupt perception of both translational and radial type motion. As this prediction aligns with the findings reported here, this local versus global processing model could be used to explain how these motion areas are processing optic flow type stimuli.

In order to be certain of the validity of these results, it was important to investigate the potential for confounding variables including: time taken to respond, bias for pressing one key over another, and speed-accuracy trade-offs. Average response time data showed no significant effects across conditions for any of the three tasks, which shows that the time taken to respond probably did not have an effect on the performance on the task because the response times did not vary according to the TMS condition. Following this, the response bias data sought to investigate whether the effects of TMS might have affected perception of one particular direction more than another and vice versa. It was also an important measure to determine whether subjects were performing as instructed (i.e. to ensure no observers pressed the same button for every trial). A lack of significant

difference from zero across all conditions and tasks reassures that a prevailing response bias was not present for any of the motion directions.

The potential for a speed-accuracy trade-off was examined using correlational analysis between response time and percent correct on each task, averaged across all four conditions. These results highlighted two negative correlations between time to respond and performance during translational and radial motion tasks. This shows that contrary to a speed-accuracy trade-off, subjects seemed to respond slower when the task was difficult which shows that if unsure on any given trial, subjects took more time to consider their response before pressing a key.

Following the success of these results determining function of TO-1 and TO-2 in the contralateral visual field, the next step will be to investigate whether the reported large receptive field size of neurons within TO-2 has any impact on the processing of stimuli within the ipsilateral visual field. Assuming TO-1 processes translational motion and TO-2 processed translational and radial motion in the contralateral side of space, it is expected that if an ipsilateral effect occurred, it would show similarities to the pattern of results shown here. This can be investigated by applying TMS to the right hemisphere during the same three direction discrimination tasks presented on the equivalent right side of space.

It will also be interesting to investigate whether area TO-2 processes all elements of radial motion. It is now known that it is responsible for processing the direction of motion (expanding/contracting), but using this direction as a way of informing our heading or self-motion perception

requires a successful identification of the position of the centre of expansion, of focus of expansion (FOE). An experiment to investigate the role of TO-2 in the perception of FOE would allow assertions of whether TO-2 is responsible for heading or whether it is contributing to a cortical network of visual motion areas responsible for analysing self-motion.

In conclusion, these results have highlighted a dissociable function of TO-1 and TO-2 in the human brain. Both TO-1 and TO-2 appear to be responsible for processing translational motion, whilst only TO-2 is responsible for contributing to the processing of radial motion. Neither area is responsible for the processing of rotational motion, suggesting an area outside of the hV5/MT+ complex may be responsible for this.

Chapter 4.2
Investigating Ipsilateral Functions of TO-1 and TO-2 During Direction Discrimination Tasks

4.2.1 Overview

It has frequently been reported that neurons in both TO-1 and TO-2 have large receptive field sizes that encroach onto the ipsilateral side of space (Dukelow et al., 2001; Amano et al., 2009; Kolster et al., 2010). In particular, neurons within TO-2 have receptive field sizes so large that neurons within this region can be independently activated by ipsilateral peripheral radial motion stimuli (see Chapter 2; Section 2.5.1). Area TO-1 is reported to have slightly smaller receptive fields than TO-2, but there is still evidence of ipsilateral involvement within this area from fMRI and TMS experiments investigating motion memory and motion detection (Slotnick and Thakral, 2011; Thakral and Slotnick, 2011). It is presumed that this ipsilateral representation is the result of interhemispheric transfer via the corpus callosum but there is also evidence of non-callosal signals (Ffytche et al., 2000).

Previous behavioural psychophysical procedures within this thesis have highlighted that TO-1 and TO-2 in the right hemisphere are responsible for processing contralateral translational motion, and TO-2 is responsible for processing contralateral radial motion (see Chapter 4.1). The next logical step was to investigate whether these areas also demonstrate causal functionality for ipsilateral stimuli. This could be investigated in one of two ways: 1) change the TMS site to the left hemisphere and continue to show

stimuli on the left, or 2) maintain magnetic stimulation of the right hemisphere but change the stimulus position to the right. For consistency and ease of comparison between experiments, we chose to change the position of the stimulus rather than the TMS site, and with the exception of stimulus position, all stimulus parameters remained consistent with those described previously.

4.2.2 Hypothesis and Aims

This experiment investigated whether TO-1 and TO-2 have any influence of processing translational, radial or rotational motion within the ipsilateral side of space.

It was predicted that TO-1 and TO-2 within the left hemisphere should still process the stimuli appropriately and therefore only small effects of TMS application to the right hemisphere would be observed. It was hypothesised that there may be a small decrease in ability on the translational task when TMS is applied to TO-1 and TO-2 and a small decrease in ability on the radial task when TMS is applied to TO-2.

4.2.3 Methods

4.2.3.1 Subjects

Six subjects (mean age 30.5 years; range 21-46 years; two female) were recruited from the University of Bradford. Four of these subjects (S2, S4, S6, S7) were naïve to the aims of the experiment and all experimental procedures were single-blind. All subjects had normal or corrected-to-normal

vision at the time of testing and had no history of neurological or psychiatric disorders. Subjects were fully informed of any possible risks associated with fMRI and TMS procedures and experiments were approved by ethics committees at both the University of Bradford and York Neuroimaging Centre. All experiments were carried out in accordance with the Declaration of Helsinki and accepted TMS safety protocols (Wassermann, 1998; Lorberbaum and Wassermann, 2000).

4.2.3.2 Identification of Target Sites

Experimental (TO-1, TO-2) and control (LO-1) target sites for the six subjects were consistent with the co-ordinates used and described in previous chapters (see Chapter 2; Table 2.2).

4.2.3.3 Psychophysical Stimuli and Procedure

The same three types of motion were tested as those described in Chapter 4.1 (translational, radial, rotational), however all stimuli were now horizontally displaced 15° (to centre) to the right of fixation as opposed to the left (Figure 4.6). The same physical characteristics of the dots applied in this experiment as in those described previously (see Chapter 2; Chapter 4.1), and the threshold levels for subjects were extracted from the contralateral presentation of the stimuli (see Chapter 2; Table 2.3). This was kept consistent to allow appropriate comparison across contralateral and ipsilateral presentations, and also because individual performance at baseline using these values produced reliable results.

Subjects were required to indicate with an appropriate keyboard press whether they perceived the coherent dots to be moving in one of two directions for each of the three motion stimuli: translational (up/down), radial (inward/outward), and rotational (clockwise/anticlockwise). Each TMS condition for each task consisted of 100 trials split over two runs.

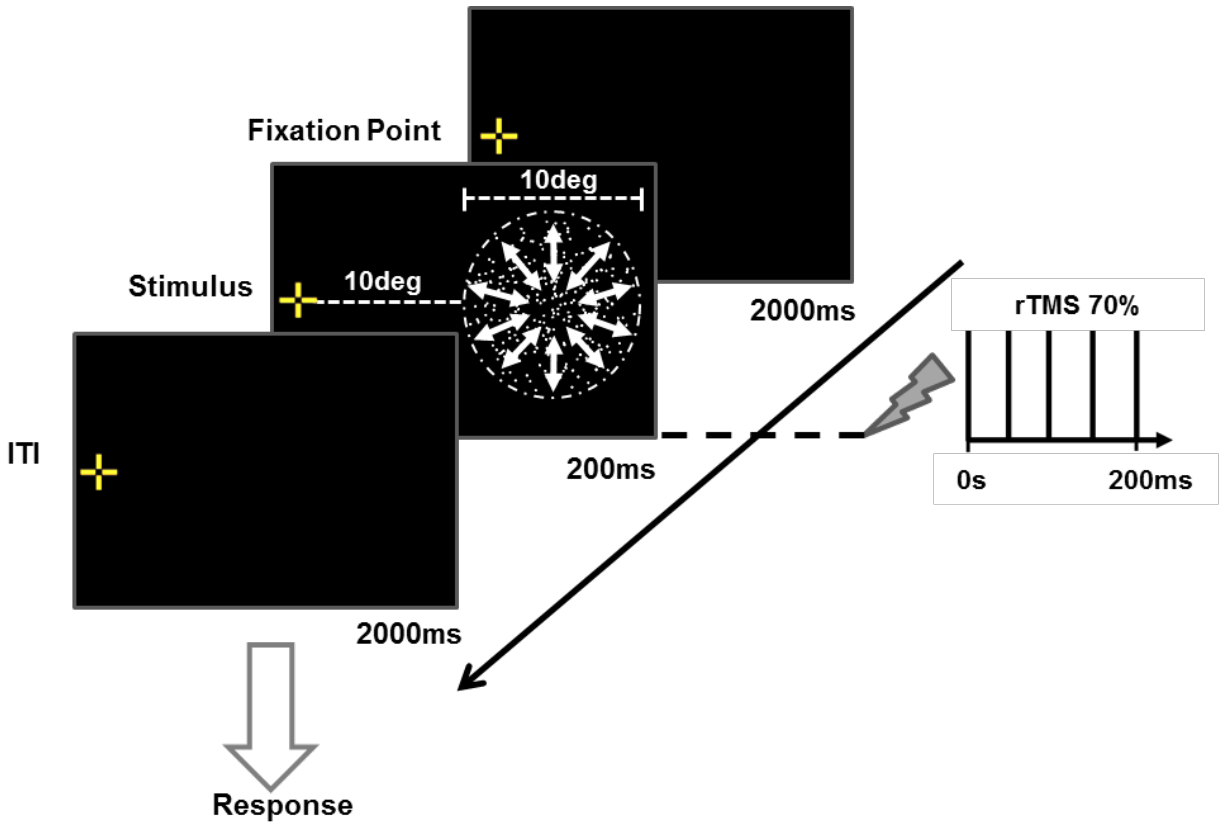

Figure 4.6. Schematic of psychophysical procedure using radial motion as an example task. There is a 2000ms ITI, in which the subject makes their response. The stimulus is shown for 200ms and onset of TMS and stimuli are synchronous. This procedure is used for three direction types: translational, radial and rotational.

Subjects viewed stimuli from a distance of 57cm with right eye occluded for all conditions.

4.2.3.4 TMS Protocol

TMS stimulation was identical to that described in Chapter 4.1 and was synchronous with the onset of the test stimulus (see Figure 4.6). The train of pulses persisted for a duration of 200ms for each trial.

4.2.3.5 Data and Statistical Analysis

Statistical analyses were carried out using IBM SPSS Statistics 20, and analyses were similar to those computed and described in Chapter 4.1. Any trials in which the TMS coil did not produce a pulse were removed from the data prior to analyses (~4%).

4.2.4 Results

4.2.4.1 Percent Correct

The primary dependent variable for this task was the percent correct (%). Repeated measures ANOVAs were applied to the data in order to determine application of TMS affected percent correct across either of the three motion direction discrimination tasks. Average values are plotted in Figure 4.7. A significant main effect was found for translational motion (sphericity assumed, $\chi^2(5) = 3.39$, $p = 0.651$; $F(3,15) = 11.67$, $p < 0.001$). Subsequent (Bonferroni corrected) pairwise comparisons revealed significant differences between Baseline and application of TMS to both TO-1 ($p = 0.008$) and TO-2 ($p = 0.042$). All other comparisons were found to be non-significant (Baseline *versus* Control, $p = 1.00$; TO-1 *versus* TO-2, $p = 0.940$; TO-1 *versus* Control, $p = 0.113$; TO-2 *versus* Control, $p = 0.301$). Radial and rotational motion

were both found to be normally distributed (radial, $\chi^2(5) = 5.14$, $p = 0.413$; rotational, $\chi^2(5) = 5.27$, $p = 0.398$) but no significant differences were identified between conditions for either the radial ($F(3,15) = 0.63$, $p = 0.609$) or rotational tasks ($F(3,15) = 0.35$, $p = 0.787$). This shows that application of TMS to TO-1 and TO-2 affects perception of ipsilateral translational direction.

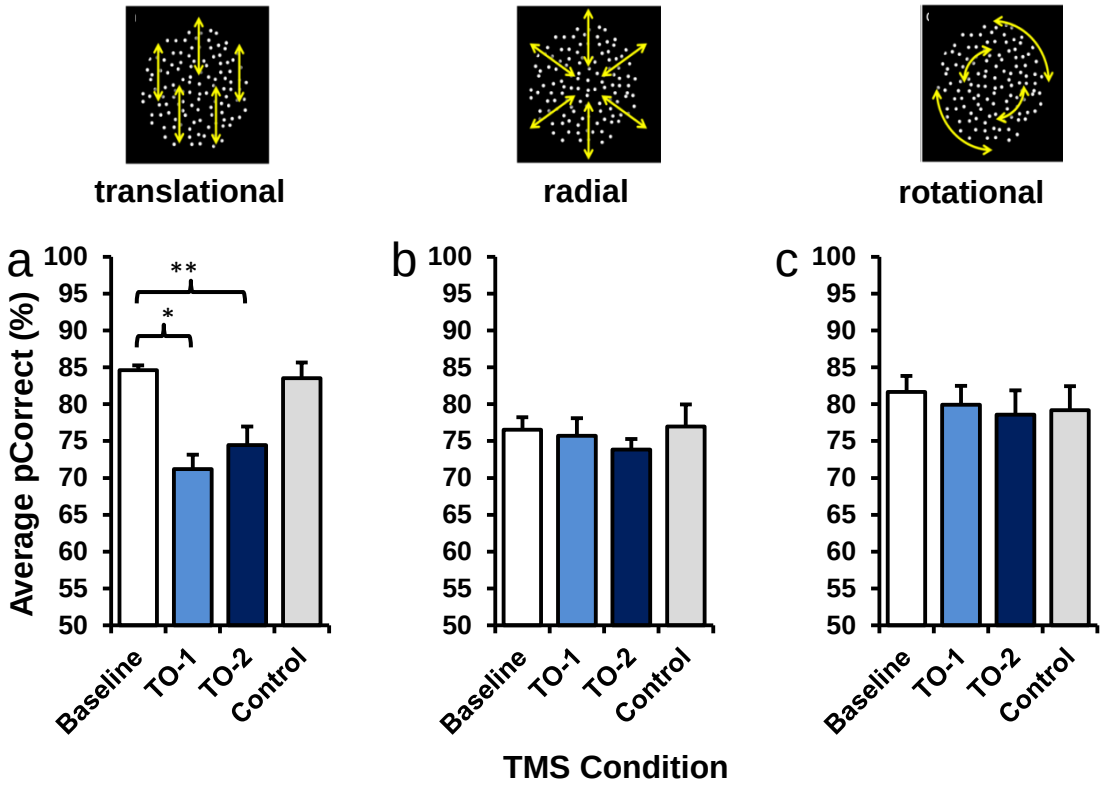

Figure 4.7. Bar charts showing percent correct (%) for each of the three tasks: translational (a), radial (b), and rotational (c). Significant differences are shown with asterisks (* = $p<0.05$; ** = $p<0.01$). Error bars represent S.E.M.

4.2.4.2 Response Times

Response times (s) were recorded in order to investigate any potential differences during application of TMS (Figure 4.8). Repeated measures ANOVA analyses revealed no significant main effects for translational (sphericity assumed, $\chi^2(5) = 6.27$, $p = 0.295$; $F(3,15) = 0.54$, $p = 0.664$),

radial (sphericity assumed, $\chi^2(5) = 6.14$, $p = 0.307$; $F(3,15) = 0.98$, $p = 0.430$) or rotational motion tasks (sphericity assumed, $\chi^2(5) = 7.16$, $p = 0.223$; $F(3,15) = 1.48$, $p = 0.261$).

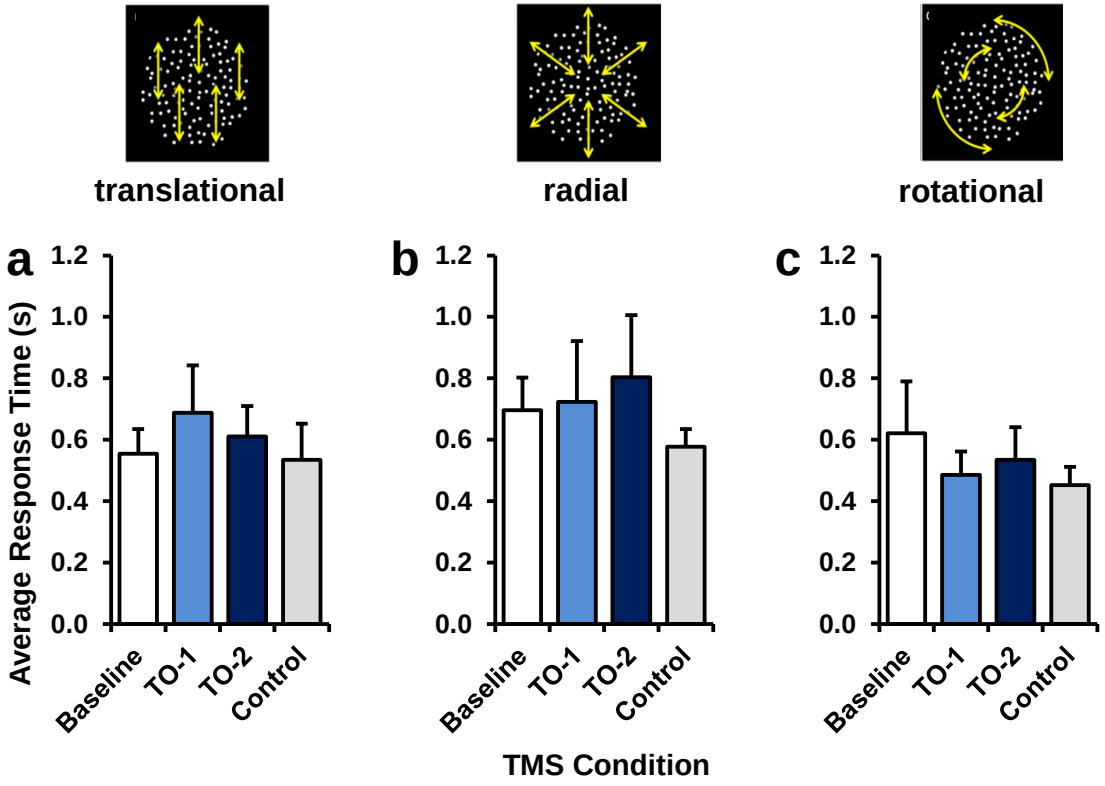

Figure 4.8. Bar charts showing average response times (s) for each of the three tasks: translational (a), radial (b), and rotational (c). No significant differences were found for any task or condition. Error bars represent S.E.M.

4.2.4.3 Response Biases

Response biases were measured to investigate whether there was any potential preference of a particular direction either with or without application of TMS (Figure 4.9). Repeated measures ANOVA analyses highlighted no significant effects across any of the three measured tasks: translational (sphericity assumed, $\chi^2(5) = 5.23$, $p = 0.403$; $F(3,15) = 0.75$, $p = 0.539$), radial (sphericity assumed, $\chi^2(5) = 6.03$, $p = 0.318$; $F(3,15) = 1.26$, $p = 0.325$)

or rotational motion (sphericity assumed, $\chi^2(5) = 7.83$, $p = 0.179$; $F(3,15) = 0.36$, $p = 0.780$).

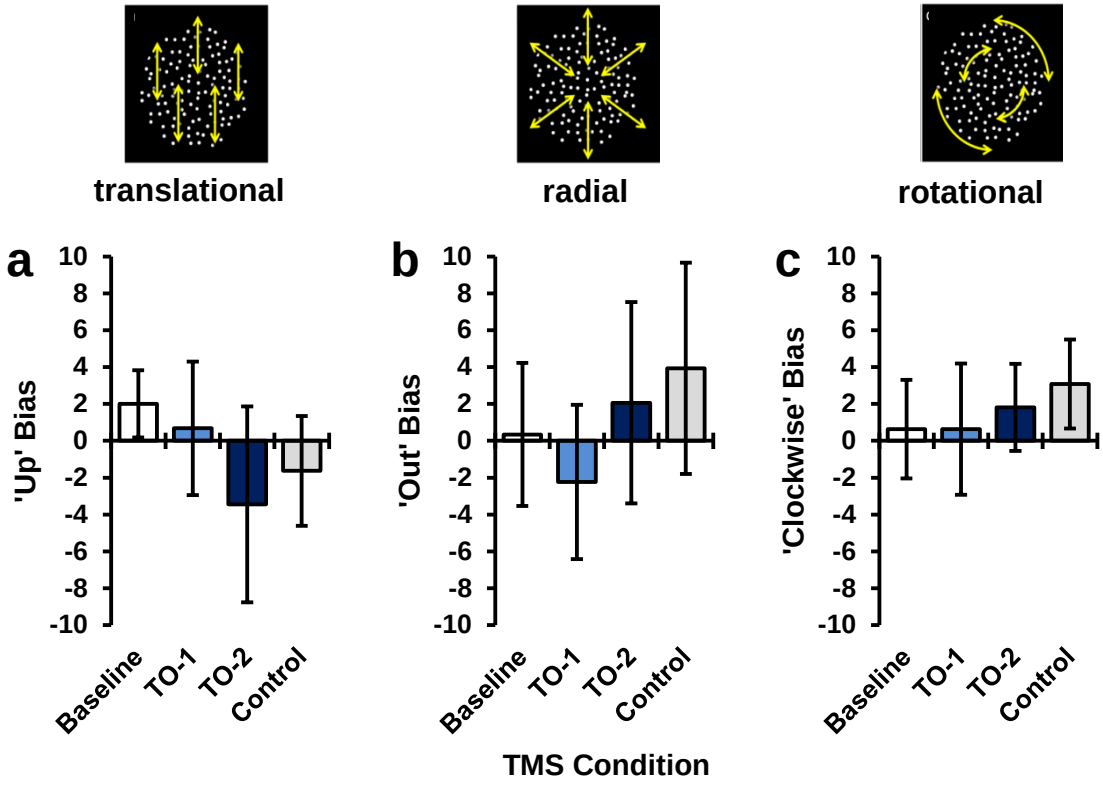

Figure 4.9. Bar charts showing average response biases for each of the three tasks: translational (a), radial (b), and rotational (c). No significant differences were found for any task or condition. Error bars represent S.E.M.

Student t-tests revealed no significant differences from 0 for any of the three direction tasks: translational (Baseline [t(5)= 1.10, $p = 0.321$]; TO-1 [t(5)= 0.18, $p = 0.859$]; TO-2 [t(5)= -0.65, $p = 0.546$]; Control [t(5)= -0.55, $p = 0.607$]), Radial (Baseline [t(5)= 0.09, $p = 0.933$]; TO-1 [t(5)= -0.53, $p = 0.617$]; TO-2 [t(5)= 0.38, $p = 0.721$]; Control [t(5)= 0.69, $p = 0.523$]), Rotational (Baseline [t(5)= 0.24, $p = 0.824$]; TO-1 [t(5)= 0.18, $p = 0.866$]; TO-2 [t(5)= 0.77, $p = 0.476$]; Control [t(5)= 1.28, $p = 0.237$]).

4.2.4.4 Speed-Accuracy Trade-Off

If subjects responded quickly at the cost of accuracy, this could negatively affect results as there would be a large proportion of errors. To investigate this, percent correct was correlated against response times (see Table 4.2). Evidence of a positive correlation would imply a speed-accuracy trade-off may be present, whereas evidence of a negative correlation would suggest that slow responses were due to harder trials.

Table 4.2. Table reporting means and standard deviations for percent correct and response times for the correlational analysis.

	Mean	Std. Deviation
Translational		
Percent Correct (%)	74.44	7.38
Response Times (s)	0.60	0.25
Radial		
Percent Correct (%)	75.78	5.19
Response Times (s)	0.70	0.33
Rotational		
Percent Correct (%)	79.83	6.65
Response Times (s)	0.52	0.24

Pearson's R analyses found no significant correlations between percent correct and response time for translational ($r = -0.29$, $n = 24$, $p = 0.171$) radial ($r = -0.36$, $n = 24$, $p = 0.084$) or rotational motion tasks ($r = 0.29$, $n = 24$, $p = 0.169$). This suggests a speed-accuracy trade-off did not occur (Figure 4.10).

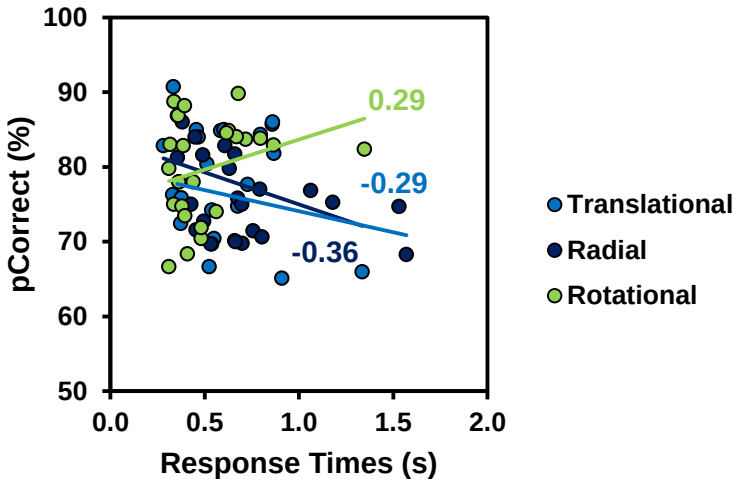

Figure 4.10. Plot showing correlation between response times (s) and percent correct (%) for each of the three tasks: translational (light blue), radial (dark blue), and rotational (green). No correlations were found to be significant.

4.2.4 Discussion

These results have shown that application of TMS to TO-1 and TO-2 in the right hemisphere produces ipsilateral deficits in performance on translational direction discrimination tasks. No effect of TMS to either TO-1 or TO-2 was found for the radial or rotational direction discrimination tasks.

This agrees with the hypothesis for translational motion but not radial motion. It was expected that application of TMS to TO-2 would also produce a behavioural deficit for radial motion as found for contralateral stimuli (see Chapter 4.1; Section 4.1.4), particularly due to the large receptive fields of neurons within TO-2 encompassing the ipsilateral side of space (Amano et al., 2009). However, it was always known that the left hemisphere would also be contributing to the processing of stimuli presented in the right hemifield as this is normal contralateral activation within left TO-1 and TO-2. This

suggests that for radial motion, the size of the receptive fields is not necessarily indicative of function. This result could also be explained by the difference between translational and radial motion highlighted in Chapter 4.1 (Section 4.1.4), as translational motion is known to utilise local motion signals whilst radial motion may result from the pooling of local translational motion signals that globally represent radially expanding/contracting motion (Yu et al., 2010). Perhaps therefore, local motion signals are able to be processed ipsilaterally within TO-1 and TO-2 whilst global motion processing requires mainly contralateral activation.

The disruption of performance observed when TMS was applied to TO-1 and TO-2 during presentation of translational motion was also unexpected as it was hypothesised that the left TO-1 and TO-2 should still be contributing to the processing of this motion. One explanation for this discrepancy could be that the right hemisphere is a dominant hemisphere for motion processing. Previous research has demonstrated that application of distal TMS (1Hz; 70%; 10 minutes) to left TO-1 (MT) impairs motion detection within the contralateral hemisphere but does not affect detection in the ipsilateral hemifield (Thakral and Slotnick, 2011). However, when TMS is applied to the right TO-1, motion detection becomes impaired in both the ipsi- and contralateral hemifields. An adapted version of their right hemisphere results can be viewed in Figure 4.11a. Also presented in Figure 4.11 are the equivalent average results from application of TMS to TO-1 in this experiment and the previous contralateral experiment. This shows the direct similarities between the two experiments despite the different motion perception tasks employed by each lab. This seems to make a clear

comparison suggesting that application of TMS to TO-1 in the right hemisphere produces ipsilateral impairments across a range of tasks. The discrepancy reported by Thakral and Slotnick (2011) between the left and right hemisphere implies a subtle dominance of the right hemisphere for motion detection, as transient disruption of the right hemisphere affects processing of the entire visual field, whilst disruption of the left hemisphere only affects the contralateral hemifield.

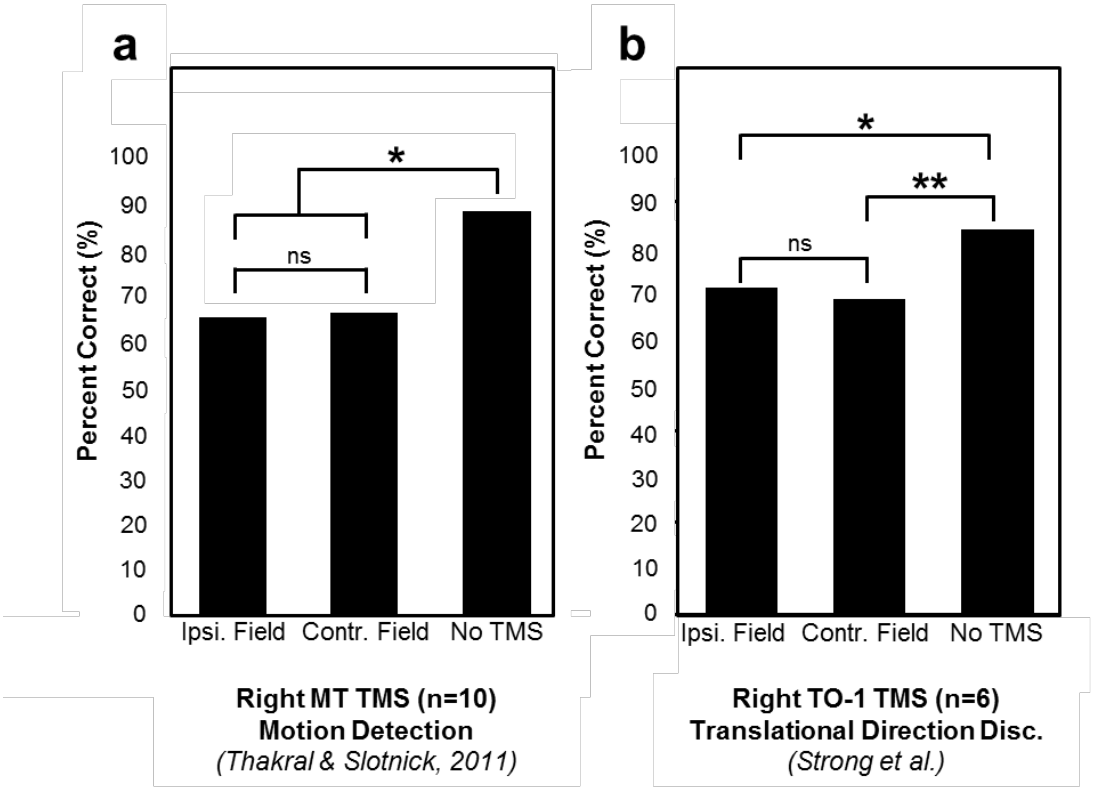

Figure 4.11. A direct comparison of percent correct when TMS is applied to TO-1 in the right hemisphere during motion detection (a) and translational direction discrimination (b). Asterisks represent significant differences between conditions (* = p<0.05; ** = p<0.01). (Adapted from Thakral and Slotnick, 2011).

These authors do discuss that the ipsilateral effects they identified may be due to transient disruption of cortical area MST (TO-2) and suggested that if localisation techniques were to be improved then they would be able to

differentiate between them (Thakral and Slotnick, 2011). However the results presented here reinforce that their localisation was likely to be accurate as the localisation employed here was specifically designed to be as precise as possible and the results are highly comparable across experiments. Also the co-ordinates used for this experiment have previously successfully functionally distinguished between TO-1 and TO-2 for contralateral radial motion perception so we are confident that application of TMS to these areas produces distinguishable effects on behaviour.

These same authors have also shown that memory for moving stimuli presented in either the left or right visual field produces measurable bilateral TO-1 fMRI activation, showing that ipsilateral responses for motion memory are also present (Slotnick and Thakral, 2011). Similarly, and more relevant for this experiment, application of TMS to TO-1 produced both contra- and ipsilateral impairments in memory for motion, further suggesting that this area is responsible for processing the ipsilateral side of space. Also, although the task described here differs from that of Slotnick and Thakral (2011), it is comparable as we are highlighting a similar ipsilateral representation of motion in TO-1 and TO-2.

There is also the possibility that TMS produces effects that spread to connected cortical regions. This has been shown to be the case between areas that rely on feed-back/forward signals within a single hemisphere as application of TMS at various onsets produces varying effects (Pascual-Leone and Walsh, 2001), but it is still not clear whether TMS can actually directly affect connected areas. For example, in this experiment, one explanation for the ipsilateral effect found for translational motion could be

that TMS applied to right TO-1 and TO-2 affects the left TO-1 and TO-2 (interhemispheric transfer). This could occur through connections known to exist across the splenium (posterior end) of the corpus callosum (Knyazeva, 2013) or other existing subcortical pathways (Ffytche et al., 2000).

Previous experiments have shown that ipsilateral signals reach hV5/MT+ slower than contralateral signals, and this is quantified as a 3ms delay in the right hemisphere compared to an 11ms delay in the left hemisphere (Ffytche et al., 2000). This is thought to correspond to a delay caused by the time taken for the signal to transfer from one hemisphere to the other via the corpus callosum as it is similar to that recorded across the corpus callosum of rhesus monkeys (Swadlow et al., 1978). This also shows that signals travelling left to right are faster than those travelling from right to left, potentially highlighting right hemispheric dominance, although the authors discuss that this may be because the stimuli always moved from left to right, as this meant right hemifield stimulation was foveopetal (towards centre) whilst left hemifield stimulation was foveofugal (away from centre). In the translational task within this experiment, motion always moved up or down so the relationship to the vertical meridian is less likely to play a role in influencing the speed of signal transfer. Ffytche et al., (2000) also recorded visually evoked potentials (VEPs) from a patient (TF) who had no posterior connection of the corpus callosum. It was found that the ipsilateral response was absent in the right hemisphere but not the left hemisphere, suggesting some ipsilateral responses may not transfer across the corpus callosum. This, along with the delay in interhemispheric transfer of normal subjects led to the conclusion that the right and left hV5/MT+ communicate across

hemispheres via both callosal and non-callosal pathways. In relation to the experiment described here, this suggests that the ipsilateral contribution of right TO-1 and TO-2 could be due to an interhemispheric transfer of information from the left hV5/MT+ complex, as a ~3ms interhemispheric delay would still be disrupted by 200ms of repetitive TMS. However, this still doesn't quite explain the lack of results found for the radial motion task, as application of TMS to TO-2 produced significant disruption for contralateral task and it would therefore be expected that similar behavioural effects would be observed for both translational and radial motion. Due to the observed disruption for translational motion when TMS is applied to TO-2, it can be assumed that the lack of effect for radial is task-specific rather than site-specific.

The lack of significant effect for response time, response bias, and speed-accuracy trade-off reasserts that the experiment was designed appropriately and results are unlikely to have been confounded by any of these secondary variables.

In conclusion, areas TO-1 and TO-2 in the right hemisphere appear to be responsible for processing ipsilateral translational motion, but neither area is necessary for processing radial or rotational motion presented within the ipsilateral field.

Chapter 4.3
Investigation of the Effect of TMS on the Psychometric Function

4.3.1 Introduction

TMS is often considered as a technique that affects the neuronal signal to noise ratio (SNR) (Walsh and Cowey, 2000; O'Shea and Walsh, 2007), however the exact impact on this SNR is still a topic of contention. Some research groups posit that it is likely to be inducing additional neural noise (Ruzzoli et al., 2010), others propose that it is acting by reducing the neural signal (Harris et al., 2008), whilst others put forward that it may be both reducing signal and increasing noise simultaneously (Allen et al., 2007). These researchers have also proposed that it may be possible to measure these effects using psychophysical procedures. In this instance the TMS would be expected to produce one of three variant effects on psychometric functions of psychophysical observers. If these theories were applied to the motion coherence paradigm tested in these experiments, it would be hypothesised that signal suppression would likely affect the point of subjective equality (PSE; bias) without affecting the curve (sensitivity) (Figure 4.12a), whereas additional neural noise would likely affect the curve without altering the PSE (Figure 4.12b). A combination of both effects would affect the curve and the PSE simultaneously (Figure 4.12c). This can be understood as an increase in noise would not affect the signal being produced; therefore the overall sensitivity to the task would be affected which in turn would result in a shallower slope but the PSE would remain unaffected. In contrast to this, if the noise remained constant but the signal

was attenuated, the PSE would be affected but the curve would remain the same. And finally, if both neural noise and signal were affected, both patterns of effect would be evident.

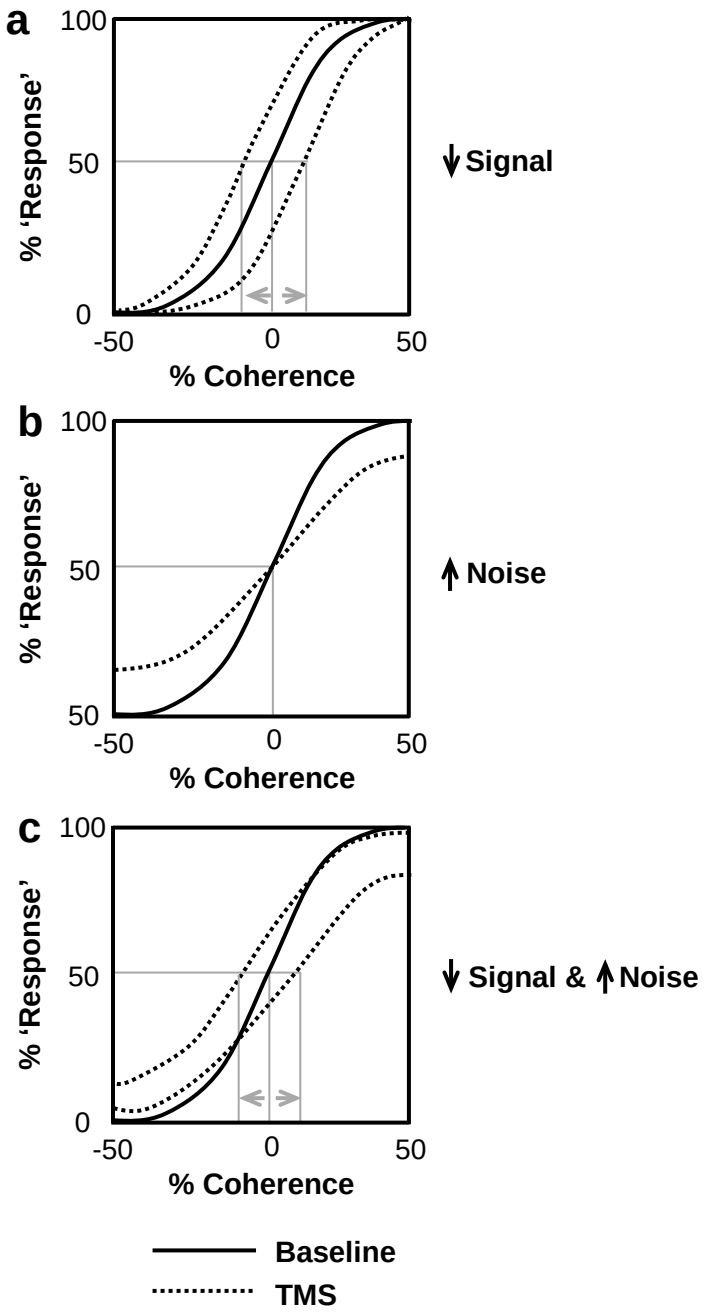

Figure 4.12. Example plots demonstrating expected pattern of effect on psychophysical data if application of TMS a) suppressed the neural signal, b) increased the level of neural noise, or c) increased noise and suppressed signal simultaneously. (Adapted from Ruzzoli et al., 2010).

Given that we have previously shown application of TMS to TO-1 affects percent correct for translational motion and application of TMS to TO-2 affects percent correct for both translational and radial motion, it followed that an investigation of the effect of TMS to these sites on the full psychometric curves for both tasks would provide further insight into whether it is more likely that TMS increases neural noise or attenuates neural signal.

4.3.2 Hypothesis and Aims

It was hypothesised that application of TMS to TO-1 and TO-2 would affect the data in one of the three potential ways: 1) increase neural noise and affect sensitivity by making the slope shallower, 2) suppress neural signal and affect bias by laterally shifting the position of the PSE, or 3) increase neural noise and suppress signal simultaneously thereby affecting the sensitivity and bias in combination.

4.3.3 Methods

4.3.3.1 Subjects

Five subjects participated in this experiment (S1,S3,S4,S6,S7; mean age 29 years; range 21-46 years; two female). Three subjects were naïve to the aims of the experiment and all experimental conditions were single-blind. All subjects had normal or corrected-to-normal vision at time of testing and had no history of neurological or psychiatric disorders.

4.3.3.2 Identification of Target Sites

For this experiment, TMS was applied to TO-1 and TO-2 in the right hemisphere of all subjects. The target co-ordinates were identical with those described previously (Chapter 2; Table 2.2).

4.3.3.3 Psychophysical Stimuli and Procedure

The psychophysical paradigm tested here was identical to that described in Chapter 4.1, the only difference being that a range of motion coherence levels (%) was tested for each condition as opposed to one individually tailored level. This allowed analysis of data along a full psychometric function. Two motion tasks were tested: translational (up/down) and radial (outwards/inwards). Conditions within the rotational motion did not produce any significant effects in Chapter 4.1 so were omitted from this experiment. Level of coherence (percentage of signal dots) ranged from 5%-50% in seven steps, and each coherence level was observed 14 times per condition, resulting in 98 total trials per condition.

4.3.3.4 TMS Protocol

For TMS conditions, repetitive TMS (25Hz; 70% strength; 50mm figure-eight coil) was applied concurrently with onset of stimuli for an equivalent duration of 200ms.

4.3.3.5 Data and Statistical Analysis

Any trials involving a response time of more than three seconds or showing a positional deviation of the TMS coil (greater than 2mm) were removed from the analysis.

For the purposes of investigating the effects of TMS on bias, the data was signed (positive or negative) depending on the direction of motion. For the translational task, upward movement was positively signed whilst downward motion was negatively signed, and for the radial task, expanding (outward) motion was positively signed, whilst contracting (inward) motion was negatively signed. PSE was measured as the point at which 50% correct crossed the x axis.

4.3.4 Results

To investigate which aspect of the signal:noise ratio (SNR) was affected by the TMS, the full psychometric curve was measured on five subjects (S1, S3, S4, S6, S7) for any sites that produced a significant effect on motion perception (i.e. TO-1 and TO-2 for translational motion and TO-2 for radial motion). The figures below show average data with fitted functions for both the translational (Figure 4.13a) and the radial tasks (Figure 4.13b). Individual data plots can be viewed in Appendix 4 and Appendix 5.

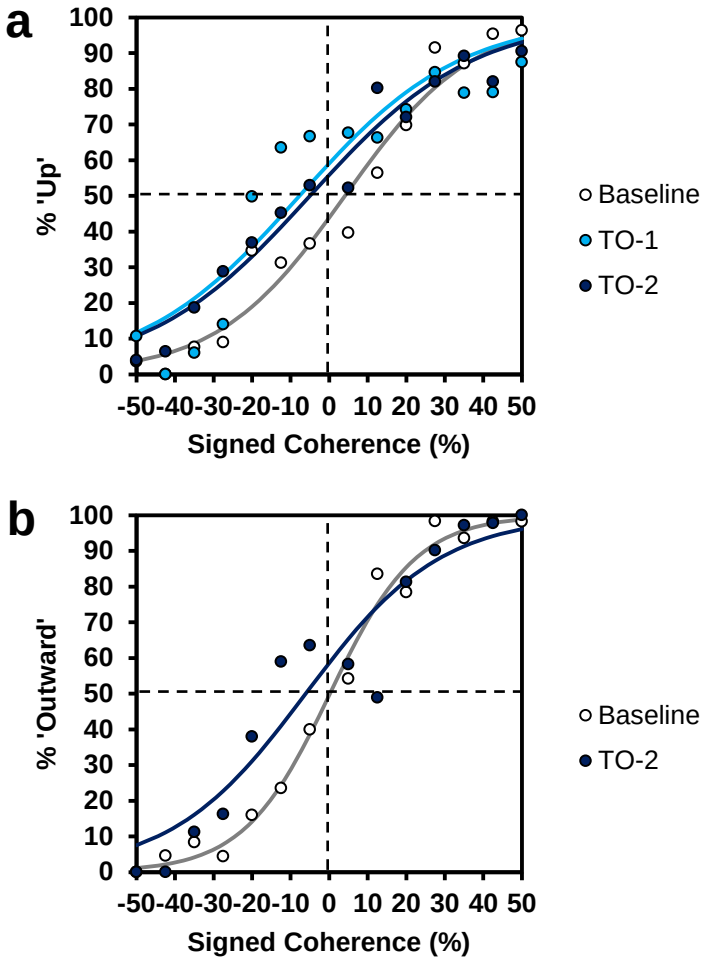

Figure 4.13. Average data (n = 5) for translational (a) and radial (b) motion tasks. These plots show the slopes become shallower for both tasks when TMS is applied to cortical areas responsible for processing those directions of motion (suggesting a decrease in sensitivity).

On visual inspection these data appear to indicate that both the bias (PSE) and sensitivity (slope) can be affected by the TMS as for experimental conditions (TO-1 and TO-2), the slope becomes shallower and moves towards the left. Further analysis of the average sensitivity (at 50%; Figure 4.14) and average bias (Figure 4.15) for each task revealed significant differences across conditions for sensitivity to the task; thereby suggesting that application of TMS has an effect on sensitivity but not bias. Repeated measures ANOVAs of the translational data revealed a significant main

effect of TMS condition on sensitivity (sphericity assumed, $\chi^2(2) = 3.88$, $p = 0.144$; $F(2,8) = 10.81$, $p = 0.005$), and subsequent pairwise comparisons (Bonferroni corrected) showed this was due to a difference existing between Baseline and TO-2 conditions ($p = 0.033$). All other comparisons failed to show significant differences (Baseline *versus* TO-1, $p = 0.159$; TO-1 *versus* TO-2, $p = 0.762$). Repeated measures ANOVAs applied to the bias data also revealed a significant main effect of condition on bias (sphericity assumed, $\chi^2(2) = 1.89$, $p = 0.388$; $F(2,8) = 6.63$, $p = 0.020$). However, pairwise comparisons (Bonferroni corrected) failed to highlight any significant differences between conditions (Baseline *versus* TO-1, $p = 0.075$; Baseline *versus* TO-2, $p = 0.128$; TO-1 *versus* TO-2, $p = 0.626$).

Paired t-tests used to analyse the radial task found a significant difference between conditions for slope ($t(4) = 3.31$, $p = 0.030$), but no significant difference for bias ($t(4) = 1.56$, $p = 0.194$).

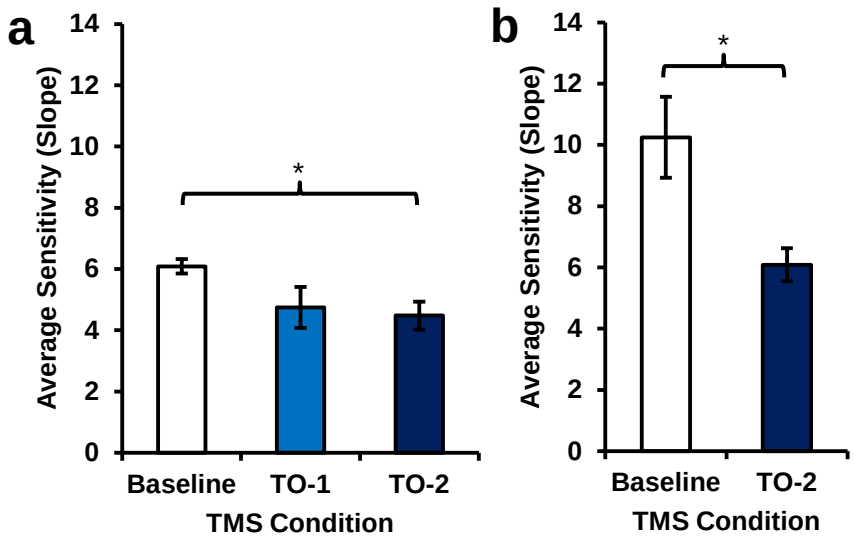

Figure 4.14. Average sensitivity (at 50%) taken from data collected across range of coherences for translational (a) and radial (b) motion tasks. Sensitivity decreases with application of TMS for both tasks (n=5). Asterisks highlight significance at $p<0.05$ (*) levels. Error bars represent S.E.M.

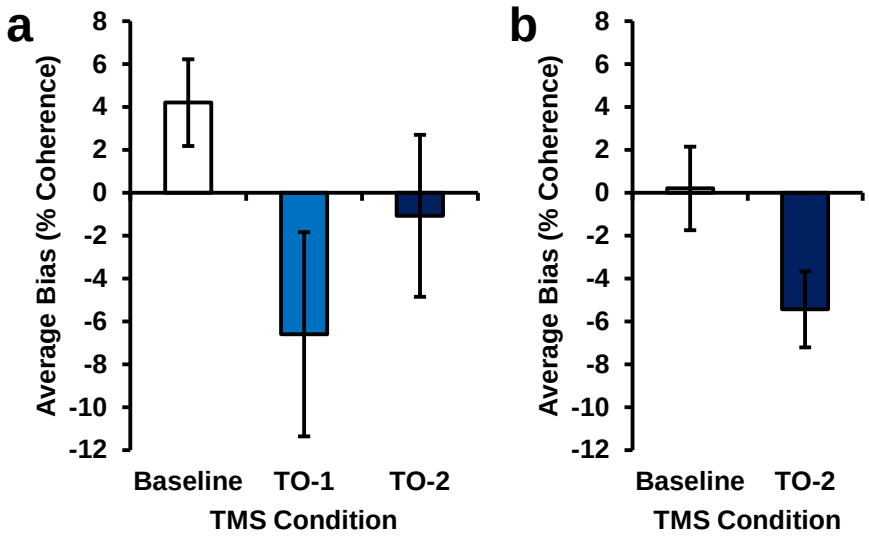

Figure 4.15. Average bias taken from data collected across range of coherences for translational (a) and radial (b) motion tasks. Bias shifts towards downward (translational) and inward (radial) motion with application of TMS for both tasks but this difference did not reach significance (n=5). Error bars represent S.E.M.

4.3.5 Discussion

Analysis of the data investigating the impact of TMS on the shape of the full psychometric curve indicated that for both translational and radial motion, application of TMS affects the sensitivity of the subject. This can be understood as fitting the model proposed by Ruzzoli et al. (2010) in Figure 4.12b (Section 4.3.1), showing that the application of TMS must be increasing neural noise within each subject.

The sensitivity data for the translational task identified a significant difference between Baseline and application of TMS to TO-2 (p = 0.033), but no significant difference between Baseline and TO-1 (p = 0.159). In theory, this result would be expected to be more similar across TMS conditions given that the overall effect on performance was similar across both conditions (see Chapter 4.1). Instead, this highlights that although TMS specifically affects ability to perform on the task, it does not have an equal power of effect on the SNR. As evident in Figures 4.13a and 4.14a, there is a very similar effect of TMS application to both TO-1 and TO-2; it just seems however that the variance was higher in the TO-1 condition which therefore reduced the power of the effect. This implies that a greater number of subjects may contribute towards producing a significant difference between Baseline and TO-1, but crucially it also suggests that TO-2 may be more important for processing translational motion than TO-1, as the TMS has a more consistent detrimental effect on sensitivity when applied to this anterior motion area.

When considering the effects of the bias the data showed no significant difference across bias data for either of the two motion tasks, although there was a significant main effect across the translational motion data. It is clear from looking at Figure 4.15 that this lack of significance is likely due to the amount of variance across conditions, but it is also important to consider that a very conservative statistical correction was used on the translational data (Bonferroni correction). This was appropriate because it reduced the possibility of a Type 1 error (identifying a false positive), however it does mean that it is more difficult for differences across the data to reach statistical significance. This means that although the bias looks to be affected, it is clearly affected in an inconsistent way between subjects which makes reaching a definitive conclusion difficult. Overall it seems that although there may be an effect of signal suppression during application of TMS, it is not one that is strong enough to produce an effect identifiable with the stringent statistical correction, perhaps due to varying degrees of effect across individuals.

When we compare these results to previous data, the findings show a significant effect of TMS on sensitivity but no significant effects on bias, thereby suggesting the data are most similar to those presented Ruzzoli et al. (2010) in which they conclude that TMS induces an increase in neural noise. However if the average plots are visually compared to the predicted models of effects of TMS in Section 4.3.1, it is clear that these data appear to be most similar to Figure 4.12c and therefore most congruent with the model presented by Allen et al. (2007). As the bias data did not reach statistical significance, no specific conclusions can be drawn, but it certainly seems

likely that there may be a small effect of TMS on bias (and therefore a small amount of signal suppression). However, this lack of significance actually contributes towards making our data more similar to those presented by Ruzzoli et al. (2010) as they also concluded that only an effect on sensitivity was evident due to there being no significant differences between conditions for bias, though the data (presented in Figure 4.16) shows that when TMS was applied to V5, there was a trend for data to shift rightward. In fact, in seven of nine subjects, a rightward shift was evident with application of TMS to V5 (Ruzzoli et al., 2010). Given that the Ruzzoli experiment did not distinguish between subdivisions of hV5/MT+ and our bias data did not reach significance, it is difficult to compare results directly, but it does seem that it is likely TMS is affecting both the bias and the sensitivity at varying levels. Certainly the effects across experiments are very similar, which reasserts that our experiment has been successful.

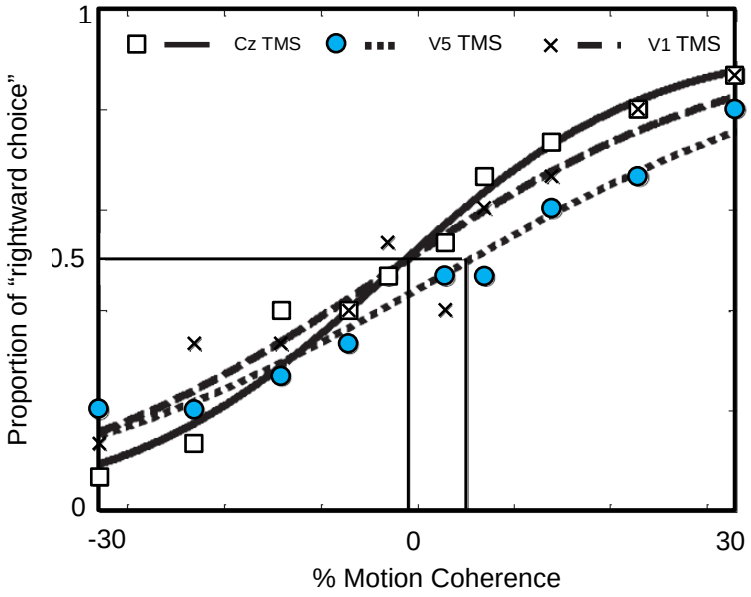

Figure 4.16. Group averaged logistic for three TMS conditions (Cz, V5, V1/V2). (Adapted from Ruzzoli et al., 2010).

In summary, when considering effect of TMS on performance, it is clear that for both translational and radial motion tasks, the application of TMS to TO-2 produced a decrease in sensitivity (thereby inferring an increase in neural noise). As this result was the same for both tasks, this demonstrates the most consistent effect of application of TMS in general (irrespective of task) was the subject finding it more difficult to distinguish between varying levels of motion coherence (less sensitive to changes).

Chapter 4.4
Investigating the Effect of Applying TMS to V3A during Direction Discrimination Tasks

4.4.1 Introduction

Previous experiments within this Chapter (4.1; 4.2; 4.3) have assessed the functions of TO-1 and TO-2 during perception of motion-coherence-defined direction discrimination tasks. However, these areas are not the only areas to be responsive to motion in the human brain, as there is also posterior occipital area V3A (Tootell et al., 1997). Area V3A has previously been shown to demonstrate an increase in fMRI response during viewing of moving objects (Kleinschmidt et al., 2002), to be responsible for contributing to the perception of stimulus speed and chromatically-defined motion (McKeefry et al., 2008; McKeefry et al., 2010) and to have a preference for incoherent (as opposed to coherent) dot motion (Pitzalis et al., 2013c). It has also been linked with a moderate amount of direction selectivity (Singh et al., 2000), so it was important to investigate whether this area also demonstrates a role in processing the direction of translational, radial and rotational motion.

With regard to these three types of motion, V3A has been linked with both radial and rotational motion processing. There is evidence that V3A shows modulations in BOLD responses when viewing expanding/contracting motion if the central focus of expansion (FOE) changes position in the visual field (Koyama et al., 2005). Indeed, Cardin et al. (2012) also identified that neurons within V3A adapt to FOE type stimuli, and when taken together these results suggest that V3A may be involved in the processing of radial

direction. Similarly, research has shown that V3A shows increases in activation for contour curvature-defined rotating motion (Caplovitz and Tse, 2007). These researchers propose that V3A must be involved in processing trackable features of object-motion in order to determine where the object is moving. As these stimuli were rotating, this suggests V3A may play a role in the processing of rotational motion.

However, area V3A is located adjacent to an area of cortex known to process motion-defined borders that is controversial within the literature for being unclear in terms of nomenclature and border distinction. This area is sometimes referred to as V3B as it shares a foveal confluence with V3A (Smith et al., 1998; Press et al., 2001), but it is also often assumed to be synonymous with kinetic occipital (KO) area (Zeki et al., 2003). However other researchers refer to any cortex lateral to V3A as dorsal V4 (V4d) which highlights the lack of clarity in nomenclature across differing research domains (Tootell et al., 1997). Despite this naming inconsistency, this informed us that when targeting V3A, it was important to ensure localisation was accurate in order to minimise the contribution of adjacent motion-selective areas within this V4d region.

To this end, TMS was applied to visual area V3A during perception of the three types of motion described above. In order to examine whether there was any effect on performance, the average percent correct for this condition was compared to previously collected baseline and control (application of TMS to LO-1) conditions from Chapter 4.1.

4.4.2 Hypothesis and Aims

It was hypothesised that V3A would be responsible for processing radial and rotational motion, and therefore that application of TMS to V3A should affect performance on the radial and rotational motion tasks.

4.4.3 Methods

4.4.3.1 Subjects

Six subjects (mean age 30.5 years; range 21-46 years; two female) were recruited from the University of Bradford. Four subjects (S2, S4, S6, S7) were naïve to the aims of the experiment and all conditions were single-blind. All subjects had normal or corrected-to-normal vision at the time of testing and had no history of neurological or psychiatric disorders. All experiments were carried out in accordance with the Declaration of Helsinki and accepted TMS safety protocols (Wassermann, 1998; Lorberbaum and Wassermann, 2000).

4.4.3.2 Identification of Target Sites

Experimental (V3A) and control (LO-1) target sites were consistent with the co-ordinates used and described in previous chapters (see Chapter 2; Table 2.2; Figure 4.17).

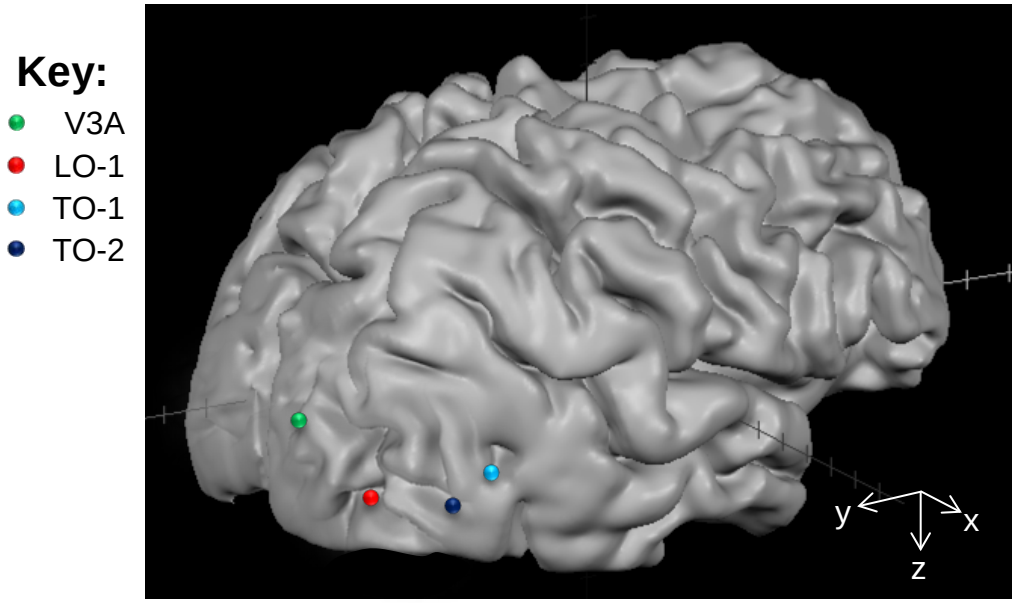

Figure 4.17. Schematic outlining position of V3A (green dot) in one representative subject (S2).

4.4.3.3 Psychophysical Stimuli and Procedure

The experimental protocol was identical to that described in Chapter 4.1. Subjects indicated the direction of 75% threshold level dots in three motion domains: translational, radial, and rotational.

4.4.3.4 TMS Protocol

The application of TMS was identical to that described in Chapter 4.1 and was synchronous with the onset of the test stimulus (see Section 4.1.3.3; Figure 4.1). The train of pulses persisted for a duration of 200ms for each trial.

4.4.3.5 Data and Statistical Analysis

Statistical analyses were carried out using statistical software (IBM SPSS

Statistics 20), and analyses were similar to those computed and described in Chapter 3 (Section 3.3.5).

Any trials in which the TMS coil did not produce a pulse were removed from the data (~4%).

4.4.4 Results

4.4.4.1 Percent Correct

The primary dependent variable was the percent correct (%). Repeated measures ANOVAs were applied to the data in order to determine whether percent correct varied across conditions within the three motion direction discrimination tasks. No significant differences were found for any of the tasks (Figure 4.18). Translational motion data points were not normally distributed ($\chi^2(2) = 15.88$, $p < 0.001$; $\varepsilon = 0.505$) and a corrected Greenhouse-Geisser analysis showed no significant effect ($F(1.01,5.05) = 0.15$, $p = 0.714$). Radial and rotational motion were both found to be normally distributed (radial, $\chi^2(2) = 4.68$, $p = 0.096$; rotational, $\chi^2(2) = 3.93$, $p = 0.140$) but no significant differences were identified between conditions for either task (radial, $F(2,10) = 0.94$, $p = 0.423$; rotational, $F(2,10) = 1.75$, $p = 0.222$).

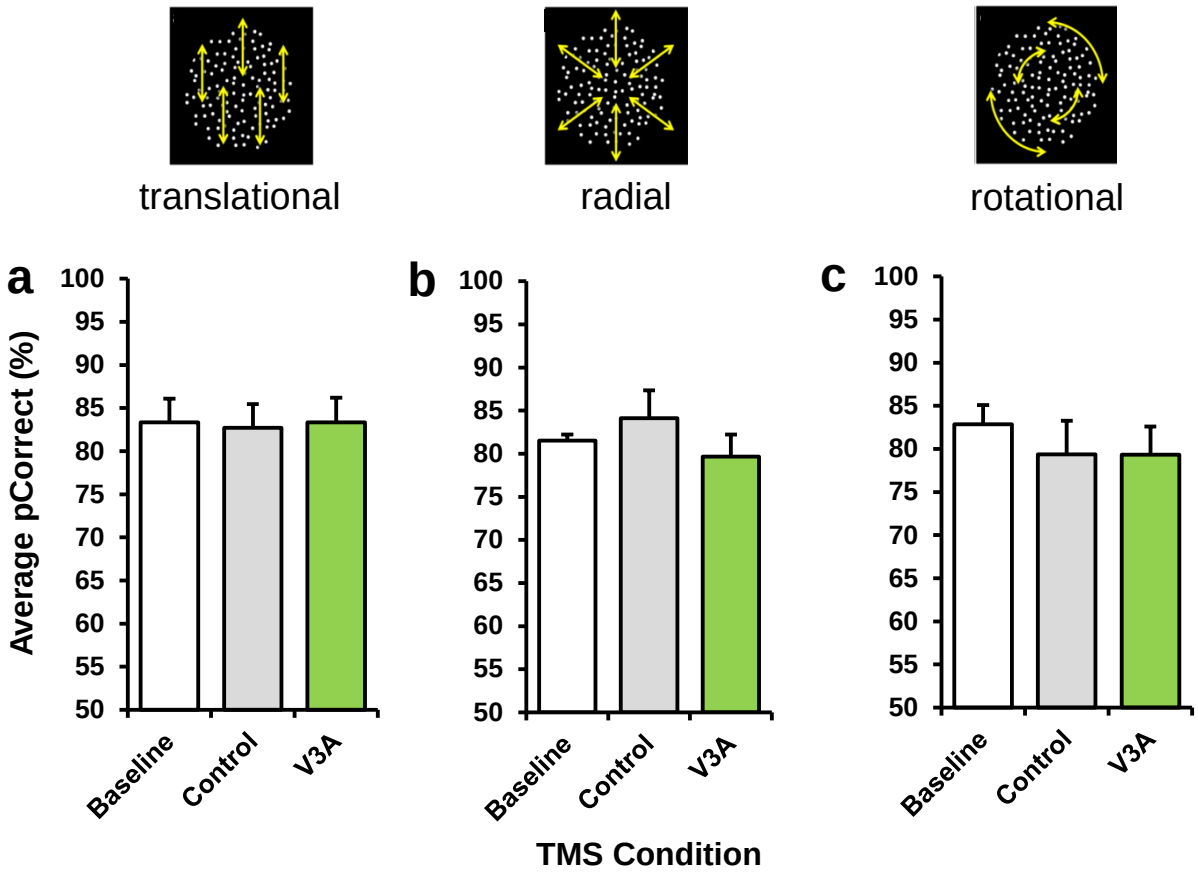

Figure 4.18. Bar charts showing percent correct (%) for each of the three tasks: translational (a), radial (b), and rotational (c). No significant differences were found for any task or condition. Error bars represent S.E.M.

4.4.4.2 Response Times

Repeated-measures ANOVAs were used to investigate whether response times varied as a function of motion task and TMS condition (Figure 4.19). These ANOVAs highlighted no significant differences between conditions for translational (sphericity assumed; $\chi^2(2) = 3.02$, $p = 0.221$; $F(2,10) = 2.40$, $p = 0.141$), radial (sphericity assumed; $\chi^2(2) = 5.18$, $p = 0.075$; $F(2,10) = 0.33$, $p = 0.726$), or rotational motion tasks (sphericity not assumed; $\chi^2(2) = 10.79$, $p = 0.005$; $\varepsilon = 0.517$; $F(1.04,5.17) = 4.61$, $p = 0.082$).

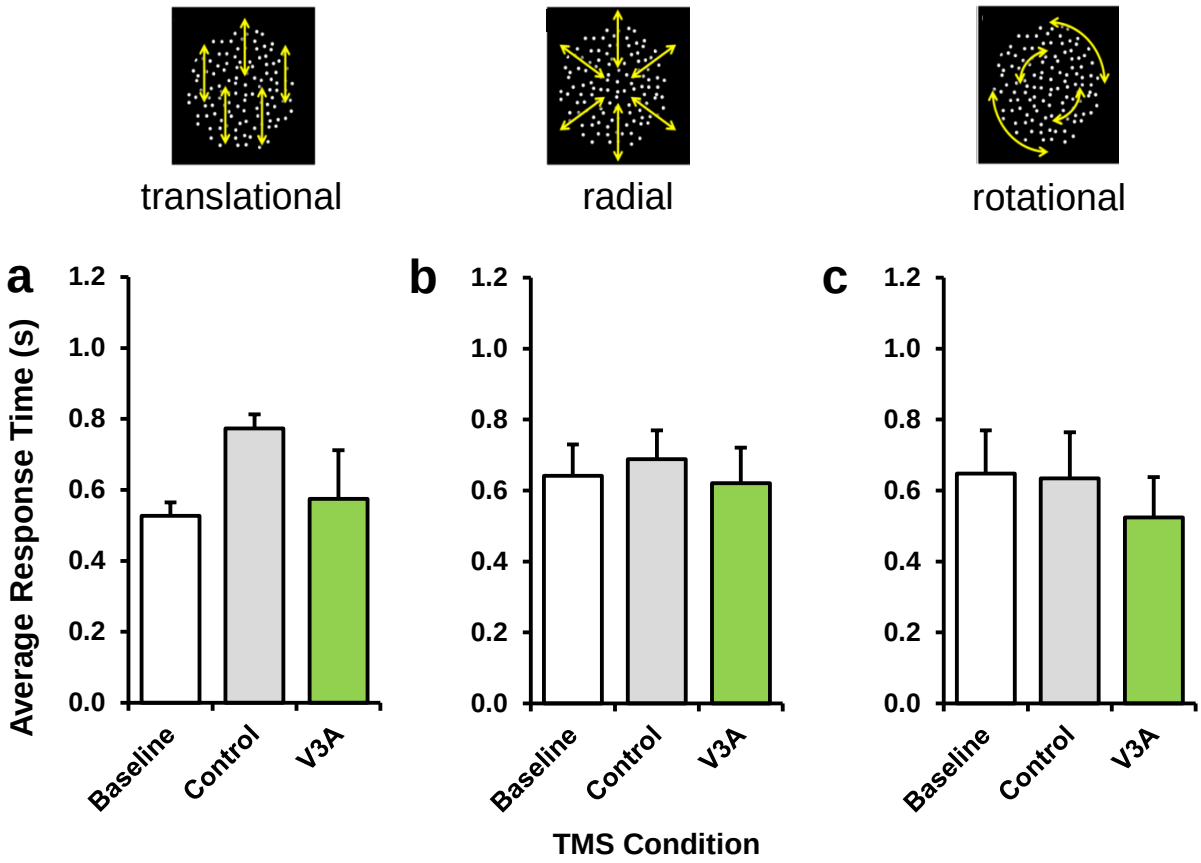

Figure 4.19. Bar charts showing average response times (s) for each of the three tasks: translational (a), radial (b), and rotational (c). No significant differences were found for any task or condition. Error bars represent S.E.M.

4.4.4.3 Response Biases

Response biases were computed using the formula described previously (see Chapter 3; Section 3.4.5; Equation 8).

Repeated-measures ANOVAs were applied to the data to investigate whether response biases existed and if so, whether they differed between conditions for each task (Figure 4.20). These analyses showed no significant differences between conditions for translational (sphericity assumed; $\chi^2(2) = 5.29$, $p = 0.071$; $F(2,10) = 0.56$, $p = 0.590$), radial (sphericity assumed; $\chi^2(2)$

= 5.99, p = 0.050; F(2,10) = 1.38, p = 0.295), or rotational motion tasks (sphericity assumed; $\chi^2(2) = 4.09$, p = 0.129; F(2,10) = 4.61, p = 0.053).

Further analyses were carried out in order to determine whether each individual condition produced a bias that significantly differed from zero (a value equating to an absence of a bias). Baseline and control conditions have already been analysed in this respect in experiment described above, so this section will focus on analysing V3A. Paired-samples t tests found no significant difference between V3A bias values relative to zero for translational (t(5)= -0.80, p = 0.462), radial (t(5)= 0.96, p = 0.383), or rotational motion (t(5)= -1.73, p = 0.145).

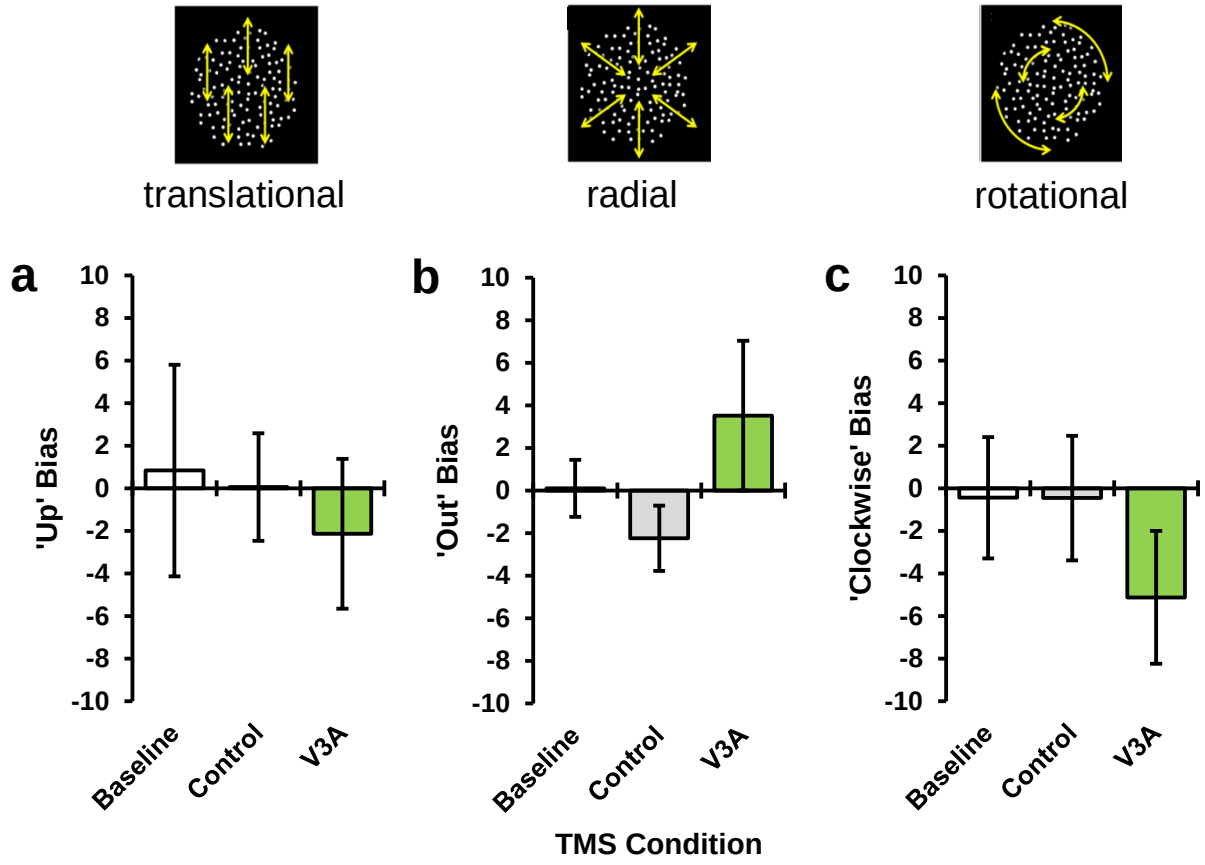

Figure 4.20. Bar charts showing average response biases for each of the three tasks: translational (a), radial (b), and rotational (c). No significant differences were found for any task or condition. Error bars represent S.E.M.

4.4.4.4 Speed-Accuracy Analysis

Correlations were analysed between response times (s) and percent correct (%) for each of the three tasks (translational, radial, and rotational) in order to investigate for any potential effects of subjects' responding quickly at the cost of accuracy (Table 4.3; Figure 4.21). Pearson's R analyses highlighted no significant correlations between response times and percent correct for translational (r= -0.29, n = 18, p = 0.251), radial (r = 0.14, n = 18, p = 0.587), or rotational motion (r = 0.27, n = 18, p = 0.278).

Table 4.3. Table reporting means and standard deviations for percent correct and response times for the correlational analysis.

	Mean	Std. Deviation
Translational		
Percent Correct (%)	83.46	6.08
Response Times (s)	0.62	0.22
Radial		
Percent Correct (%)	81.76	5.19
Response Times (s)	0.65	0.21
Rotational		
Percent Correct (%)	80.72	6.91
Response Times (s)	0.60	0.29

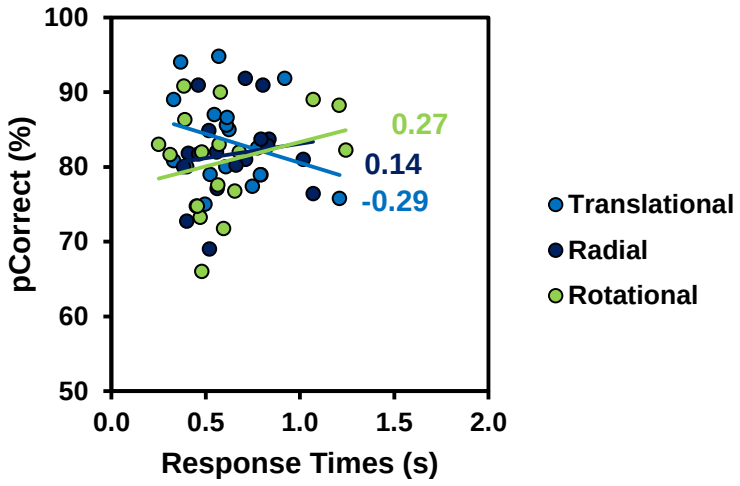

Figure 4.21. Plot showing correlation between response times (s) and percent correct (%) for each of the three tasks: translational (light blue), radial (dark blue), and rotational (green). No correlations were found to be significant.

4.4.5 Discussion

These results demonstrate that area V3A is not necessary for the processing of translational, radial or rotational motion direction which rejects the original hypothesis proposing that V3A would be involved in the perception of radial and rotational directions. This may be due to the reported preference for incoherent (as opposed to coherent) motion stimuli (Pitzalis et al., 2013c), as this task relied on appropriate perception of the coherent (signal) dots, rather than the incoherent (random) dots. However, this data appears to disagree somewhat with neuroimaging evidence presented by Koyama et al. (2005), showing that activity in V3A modulates with changes in position of the FOE of radial motion, and it also contradicts the data highlighting the role of V3A in rotating contour curvature (Caplovitz and Tse, 2007). In contrast to the proposal that V3A may be processing radial and rotational motion, the data presented here suggests that the reported change in BOLD signal from

Koyama et al. (2005) and Caplovitz and Tse (2007) may be solely the product of V3A processing the position of the FOE and the moving contours respectively, not the specific direction of the motion. Further causal investigations of whether V3A is responsible for processing FOE and motion-defined contours will be necessary in order to provide conclusive evidence to support this hypothesis (see Chapter 5).

In contrast to this, previous Chapters in this thesis (4.1; 4.2) have highlighted the role of TO-1 and TO-2 in the perception of translational and radial motion. The data presented in this chapter supports the notion that if V3A is involved in the processing, as demonstrated by increases in BOLD signal for translational, radial and rotational motion (Smith et al., 2006), then it must not be necessary as it is still possible for subjects' to perform on the task without access to appropriate signals from this area. Instead, for rotational motion, the signal must be processed in an area external to TO-1, TO-2 and V3A that likely receives input from these areas. However for radial motion, it must be the case that TO-2 is responsible for the production of the radial signal, whilst V3A uses signals produced by radial motion to determine positional information. It follows therefore, that the next empirical question to be answered would revolve around the contribution of V3A in the localisation of the FOE position.

With regards to the function of V3A, the null data presented here has suggested that the overall functional specificity of this area may be more closely aligned with interpreting and analysing textural and informative (trackable) motion information (Koyama et al., 2005; Caplovitz and Tse, 2007). This would coincide more closely with the properties of monkey V3A,

although in the monkey V3A is responsible for textural analysis of shapes, not motion (see Massot and Lee, 2014). If this is indeed the case, this would mean the dot stimuli used in these experiments might not have been suitable for selectively distinguishing the function of V3A. In the future, experiments investigating more than simple direction discrimination tasks (i.e. positional information or form-from-motion) may elicit more of an effect when TMS is applied to V3A.

Secondary variables including: response time, response bias comparisons and speed-accuracy correlations, when compared across conditions, were all found to be non-significant. This further supports the claim that application of TMS to V3A does not affect any aspect of direction discrimination for either of these three motion types.

In summary, this study has provided causal evidence to show that V3A is not responsible for processing translational, radial or rotational motion directions.

Chapter 5
Effects of Application of TMS to V3A and hV5/MT+ on Processing the Position of Focus of Expansion

5.1 Introduction

When considering the characteristic features of radial motion, the direction evidently plays a key role, but the central focus of expansion (FOE) is also an important factor for processing heading and self-motion (Wall and Smith, 2008). The combination of radially expanding/contracting motion with the known positional FOE information informs the direction of the self, which is crucial for navigating the visual environment safely and appropriately.

Previous work within this thesis (Chapter 4.1) has shown that area TO-2 is necessary for the perception of radial direction (expansion/contraction). This is consistent with neuropsychological studies which have shown that damage to hV5/MT+ (encompassing TO-1 and TO-2) leads to patients being unable to accurately identify the direction of radially moving stimuli (Beardsley and Vaina, 2005a). However, in this study, Beardsley and Vaina demonstrate that the patient's ability to identify the FOE at the centre of the radial motion remains intact. This is supported by evidence suggesting application of TMS to hV5/MT+ does not disrupt performance on a spatial positioning task involving a moving target (Campana et al., 2006). Taken together, these data suggest that FOE position must be encoded and perceived via neural activity outside of the hV5/MT+ complex. A separate motion area, V3A, has been identified as a possible cortical locus for the analysis of FOE position and it has already been shown that neurons within this area demonstrate

differential fMRI responses to changes in position of FOE stimuli (Koyama et al., 2005). This response selectivity makes it likely V3A is important for the processing of the central radial positional information. However, more recent fMR-adaptation work has proposed that all three of these areas (TO-1, TO-2 and V3A) are involved in the processing of FOE (Cardin et al., 2012), and there is prior TMS evidence demonstrating that only hV5/MT+ seems to show selectivity for FOE (Harvey et al., 2010). However the localisation of the V3A site used in this TMS experiment was not as accurate as the technique described here, and neuroimaging evidence only asserts a correlation, not causality. It was therefore important to provide causal evidence to determine which (if any) of these areas were necessary for this task, and to ensure validity of results by localising the areas for TMS consistently across individuals.

In a broader context concerning processing of self-motion; if hV5/MT+ was found not to be responsible for processing FOE location, then it could be concluded that self-motion must be processed across a network of cortical areas. Indeed, previous research has already implicated the role of V6 in the integration of depth and flow signals further up the visual pathway (Cardin and Smith, 2011), suggesting the potential involvement of this area as well.

5.2 Hypothesis and Aims

The purpose of this study was to investigate the roles played by the two sub-divisions of hV5/MT+ (TO-1, TO-2) and V3A in perception of the central focus of radial motion stimuli. Based on neuropsychological work, it was

hypothesised that application of TMS to area V3A would produce a significant reduction in subjects' performance on the FOE localisation task. Given the case study reported by Beardsley and Vaina (2005a), it is also hypothesised that application of TMS to TO-1 and TO-2 will produce no effect on performance on this task.

5.3 Methods

5.3.1 Subjects

Six subjects (mean age 30.5 years; range 21-46 years; two female) were recruited from the University of Bradford. Four of these subjects (one female) were naïve to the aims of the experiment (S2, S4, S6, S7) and all experimental procedures were single-blind. All subjects had normal or corrected-to-normal vision at the time of testing and had no history of neurological or psychiatric disorders. Subjects were fully informed of any possible risks associated with fMRI and TMS procedures, and were required to read an information sheet outlining the exact protocol of the experiment and how to participate in the experiments safely. Subjects signed a health questionnaire before every testing session.

Experiments were approved by ethics committees at both the University of Bradford and York Neuroimaging Centre, and were carried out in accordance with the Declaration of Helsinki and accepted TMS safety protocols (Wassermann, 1998; Lorberbaum and Wassermann, 2000).

5.3.2 Identification of Target Sites

Experimental (TO-1, TO-2, V3A) and control (LO-1) target sites for the six subjects were consistent with the coordinates described in previous chapters (see Chapter 2; Table 2.2).

5.3.3 Psychophysical Stimuli and Procedure

For this 2AFC task, subjects were required to press a button on the keyboard (up arrow or down arrow) to indicate whether a 'test' aperture of radially moving dots (50% inwards, 50% outwards) moved upwards or downwards (relative to a 'reference' aperture presented in horizontal alignment with fixation cross) within a field of incoherently moving dots. This coherent test aperture measured 10° within a field of incoherent dots that filled the left half of the monitor (30° x 20°). Individual dots measured ~0.2° and moved at 7°/s for 200ms per trial. Prior to performing on the experimental task it was necessary for the subjects to perform the same task across a range of positional changes (-1° - +1°) in order to determine each subjects' individual threshold level.

This threshold level was determined by fitting a psychometric function to the raw data corresponding to the number of times the subject responded 'down' (see Figure 5.1). This function extracted the distance located at the position corresponding to 50% Response Down, along with the distances at the 25% and 75% positions. The 50% position identifies the point at which the change in positions is perceived to be 0, whereas the 25% represents the threshold for identification of the upward positional change, and the 75% represents the threshold for the downward positional change.

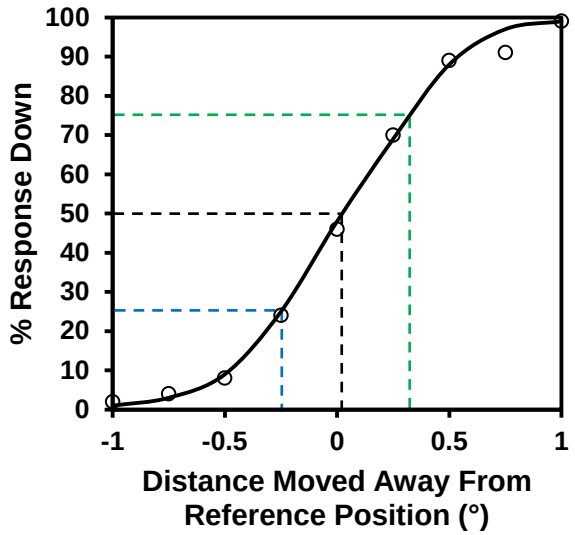

Figure 5.1. Plot showing psychometric data from example subject. The psychometric curve has been fitted using MATLAB. The black dashed line shows the extracted 50% value, the green dashed line shows the extracted 75% position and the blue dashed line represents the 25% value.

These values could have been transferred verbatim into individual MATLAB codes for the TMS part of the experiment, but if the data was skewed in any way (either leftwards or rightwards of 0), this would have led to biased values i.e. one might be closer to 0 and therefore more difficult (see Figure 5.2). To solve for this, the 75% and 25% values were both made positive and then summed in order to get the range across 0 (i.e. 0.2+0.3=0.5). This range was then divided by 2 (i.e. 0.5/2=0.25) in order to get a corrected positive (+x) and negative (-x) value to apply to the zero. This would mean that for any given trial in the experiment, the test FOE would either be positioned +x above the reference position or -x below it and these values would vary across individuals.

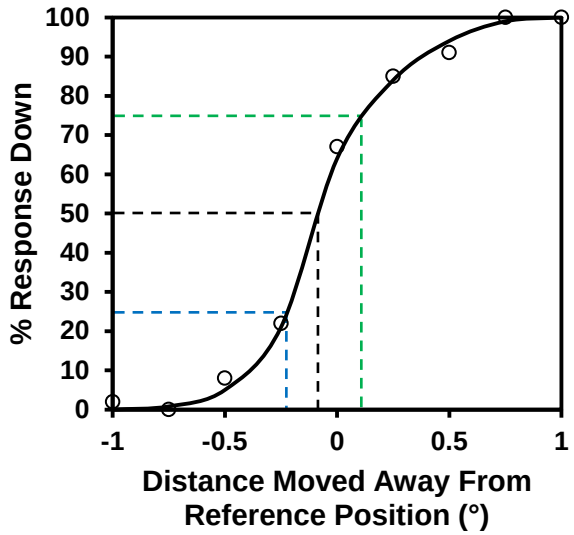

Figure 5.2. Plot showing hypothetical psychometric data. In this instance the curve is skewed to the left of the 0 making the 25% and 75% values unevenly spaced around 0. The black dashed line shows the extracted 50% value, the green dashed line shows the extracted 75% position and the blue dashed line shows the 25% value.

Once these corrected threshold values had been determined (see Table 5.1), the subjects performed the same task, but this time only viewing their threshold level of positional change (50 trials per run; total two runs per condition). The experimental conditions consisted of no TMS (baseline), TO-1, TO-2, V3A and LO-1 (control site). Subjects sat at a viewing distance of 57cm with left eye occluded for all conditions. This positioning was maintained using a chin rest.

5.3.4 TMS Protocol

Five biphasic TMS pulses were applied concurrently with onset of test stimuli at a frequency of 25Hz and a strength of 70% for 200ms (see Figure 5.3). No TMS was applied during presentation of the reference in order to allow subjects to be able to make an informed comparison.

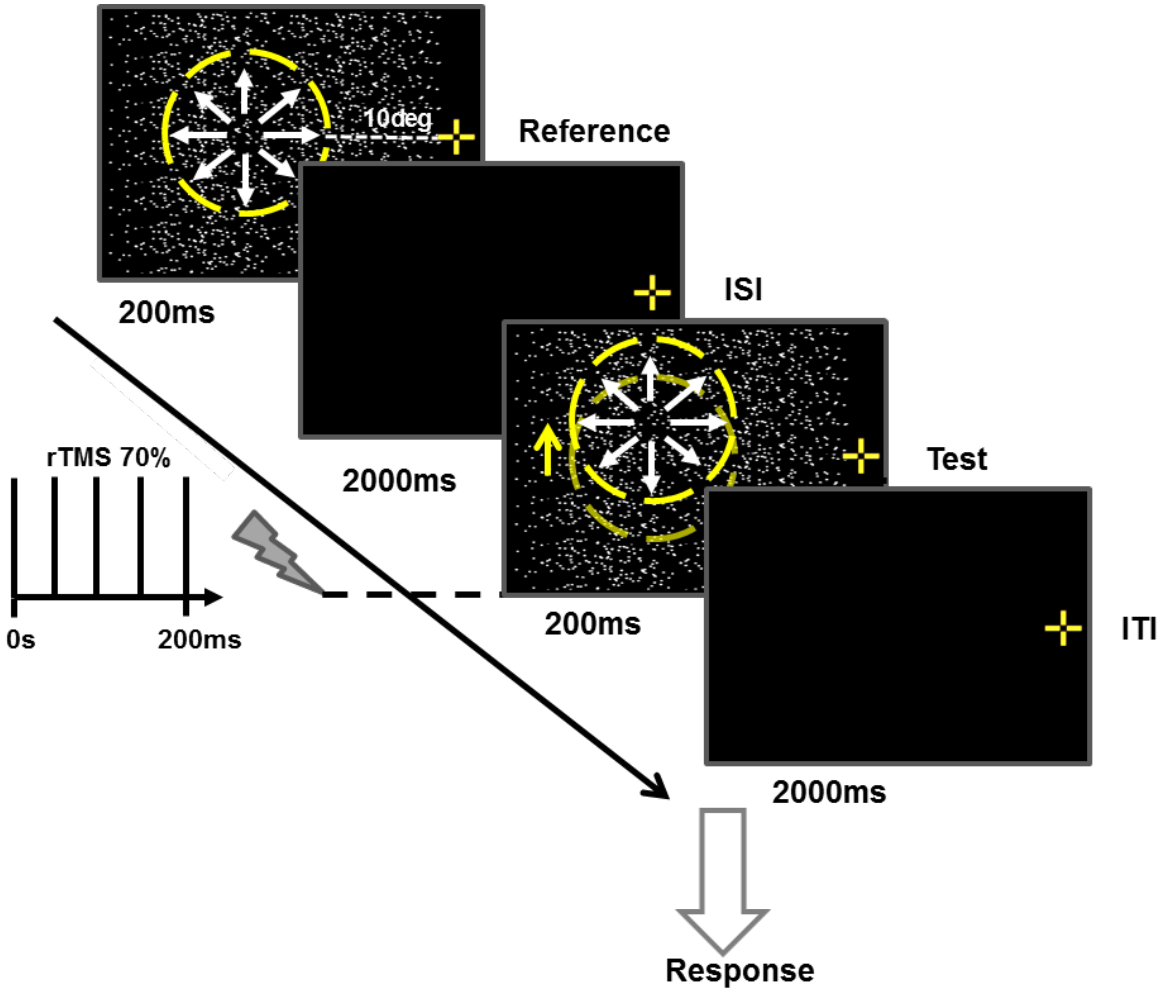

Figure 5.3. Schematic of psychophysical procedure. The reference stimulus is shown for 200ms, then the test is shown following a 2000ms ISI. The subject is required to make a response during the ITI. The onset of TMS and test stimuli are synchronous.

5.3.5 Data and Statistical Analysis

Statistical analyses were carried out using IBM SPSS Statistics 20, and analyses were similar to those computed and described in Chapter 3 (Section 3.3.5). A small percentage of trials (~5%) were removed prior to statistical analysis if the TMS coil did not produce a pulse, thereby producing an invalid trial.

5.4 Results

Individual threshold level changes in position were computed and identified using the method described above (Chapter 5.3.3), and varied between subjects across a small range of ±0.273° - ±0.440° degrees of visual angle (Table 5.1).

Table 5.1. Table showing individual 75% thresholds for FOE task in degrees of visual angle. Table also demonstrates average values (standard deviation in parentheses).

Subject	Threshold Change in Position (°)
S1	± 0.273
S2	± 0.285
S3	± 0.320
S4	± 0.440
S6	± 0.315
S7	± 0.298
Average	± 0.322 (0.06)

5.4.1 Percent Correct

Average percent correct (%) data for all five TMS conditions were plotted in Figure 5.4. A repeated-measures ANOVA (sphericity assumed, $\chi^2(9) = 17.29$, $p = 0.067$), was applied to investigate potential effects of TMS condition on performance. The analyses demonstrated a significant main effect of TMS site on percent correct on the FOE task ($F(4,20) = 12.19$, $p < 0.001$). Subsequent pair-wise comparisons (Bonferroni corrected) highlighted one significant difference between Baseline and V3A ($p = 0.009$), highlighting the important role of V3A in FOE processing. No other comparisons were

found to be significantly different (TO-1 *versus* TO-2, $p = 0.799$; TO-1 *versus* V3A, $p = 0.069$; TO-2 *versus* Control, $p = 0.217$; TO-2 *versus* V3A, $p = 0.713$; Control *versus* V3A, $p = 0.079$). All other comparisons equated to $p = 1.00$.

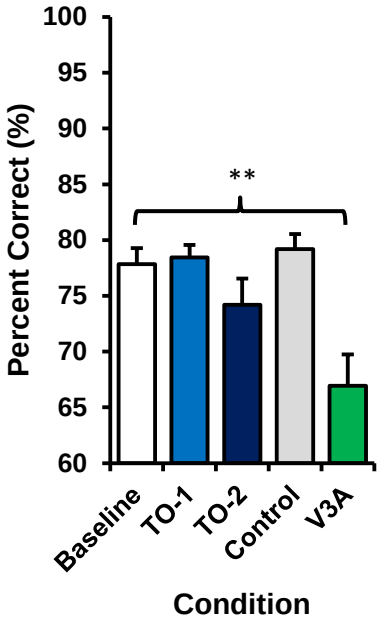

Figure 5.4. Bar charts showing average percent correct (%) across five TMS conditions for FOE task. Error bars represent S.E.M. Asterisks highlight significant difference at p<0.01 level.

5.4.1.1 Double Dissociation between TO-2 and V3A

Further analysis of this data requires a comparison between tasks. In this instance, V3A was found to be significantly different to baseline for the focus of expansion (FOE) task, but V3A was previously not associated with the perception of radial direction (see Chapter 4.4). A modified version of the figure displaying the results from Chapters 4.1 and 4.4 compared with data from this experiment can be seen in Figure 5.5.

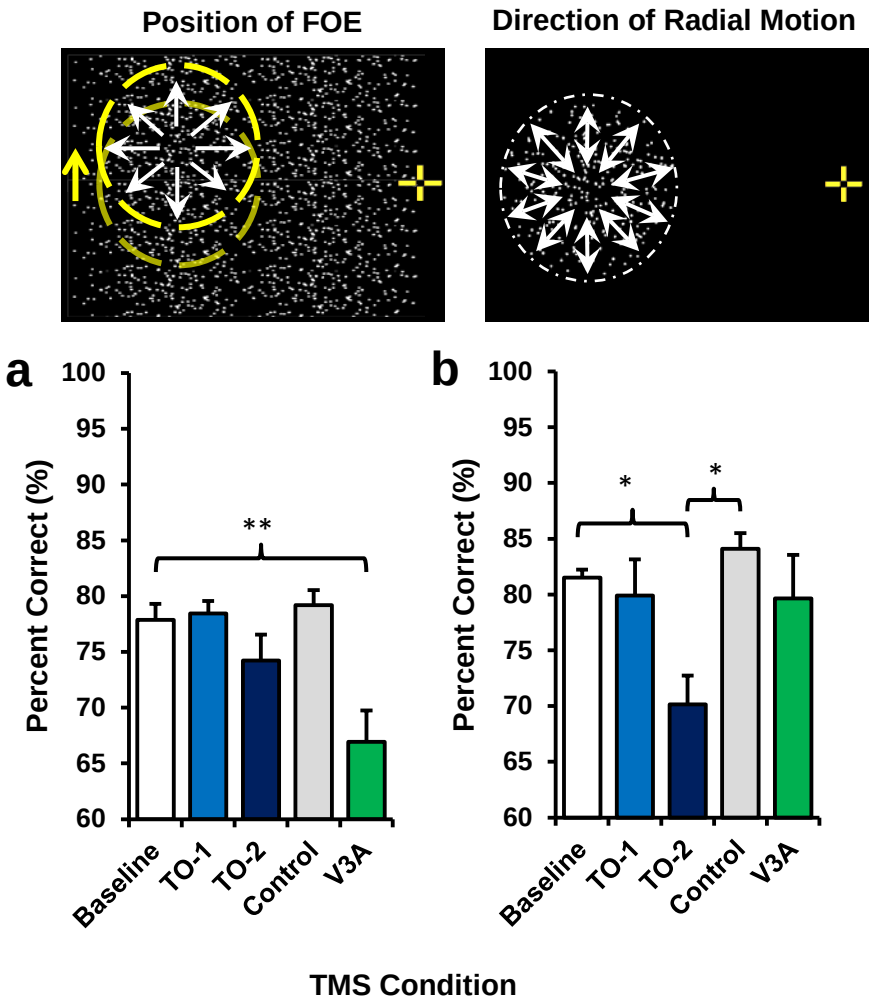

Figure 5.5. Bar charts showing average percent correct (%) across two tasks (focus of expansion (a) and radial direction (b)). Error bars represent S.E.M. Asterisks represent significance at $p<0.05$ (*) and $p<0.01$ (**).

A two-way ANOVA analysis investigating differences across the two tasks highlighted a significant interaction between TMS site and radial task ($F(4,50) = 3.48$, $p = 0.014$), showing that V3A and TO-2 must be processing these tasks independently of one another. A significant main effect was found overall ($F(9,50) = 5.21$, $p < 0.001$), and significant differences were found across tasks ($F(1,50) = 6.57$, $p = 0.013$) and TMS sites ($F(1,50) = 6.60$, $p < 0.001$). This shows that results were significantly different between tasks and TMS conditions. Subsequent pair-wise comparisons (Bonferroni

corrected) identified significant differences between TMS conditions (Baseline *versus* TO-2, $p = 0.021$; TO-1 *versus* TO-2, $p = 0.038$; TO-2 *versus* Control, $p = 0.001$; Control *versus* V3A, $p = 0.007$). All other comparisons equated to $p = 1.00$ or were non-significant (Baseline *versus* V3A, $p = 0.080$; TO-1 *versus* V3A, $p = 0.139$).

5.4.2 Response Times

Average response times (s) were plotted in Figure 5.6 and were analysed to investigate potential differences between TMS conditions. A repeated-measures ANOVA (sphericity assumed, $\chi^2(9) = 2.58$, $p = 0.982$), demonstrated no significant effects of TMS site on speed of response during the FOE task ($F(4,20) = 0.84$, $p = 0.517$).

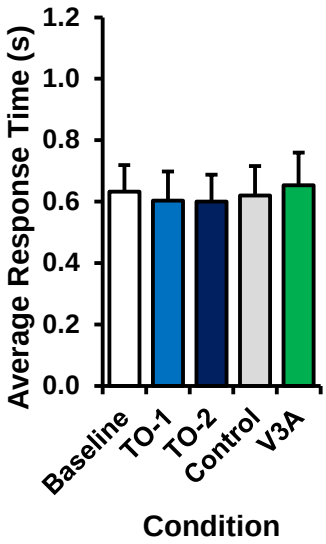

Figure 5.6. Bar charts showing average response times (s) for the FOE task. Error bars represent S.E.M.

5.4.3 Response Biases

Analysis of response bias was computed to determine whether subjects were performing appropriately on the task (0 equates to no bias), and also to confirm that application of TMS did not produce a perceptual bias in any particular direction. These values were computed using the formula described in Chapter 3; Section 3.4.5; Equation 8 (Figure 5.7). As sphericity was assumed ($\chi^2(9) = 11.18$, $p = 0.317$), a repeated-measures ANOVA reported no significant effect of TMS condition on response bias, confirming that bias was consistent across conditions ($F(4,20) = 0.59$, $p = 0.674$).

Student t-tests compared bias values with 0 (no bias) as a significant difference would indicate that performance for that condition was likely to be biased towards perceiving the directional change as upward (as the data is positive). A significant variation from zero was reported for application of TMS to TO-1 ($t(5) = 3.22$, $p = 0.023$). All other conditions were found not to be significantly different to 0 (Baseline, $t(5) = 1.49$, $p = 0.196$; TO-2, $t(5) = 1.48$, $p = 0.198$; Control, $t(5) = 1.07$, $p = 0.336$; V3A, $t(5) = 0.40$, $p = 0.705$).

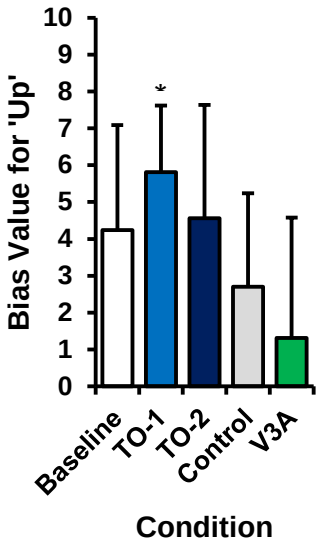

Figure 5.7. Bar charts showing average response biases for the FOE task. Positive values show on average subjects were more likely to perceive an upward shift. Asterisk represents significance at $p<0.05$. Error bars represent S.E.M.

5.4.4 Speed-Accuracy Trade-Off

Investigating the potential of a speed-accuracy trade-off is important for determining whether subjects were likely to be responding quickly and therefore potentially making more errors. This would present itself as a positive correlation as short response times would lead to less percent correct. Bivariate Pearson's R analysis identified a significant moderate negative correlation between response time and percent correct ($r = -0.37$, $n = 30$, $p = 0.047$). This shows that a speed-accuracy trade-off did not occur (Figure 5.8; Table 5.2).

Table 5.2. Table reporting means and standard deviations for percent correct and response times for the correlational analysis.

	Mean	Std. Deviation
Percent Correct (%)	75.32	6.35
Response Time (s)	0.62	0.22

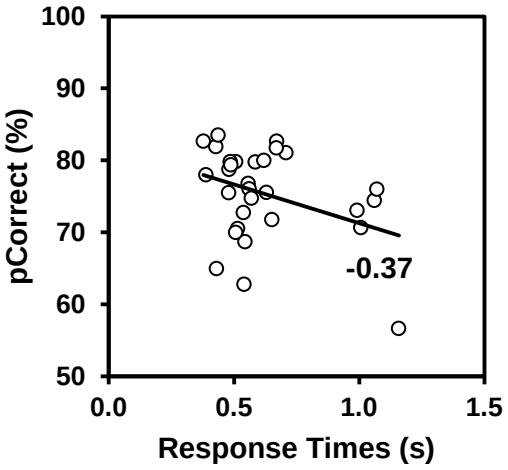

Figure 5.8. Scatter plot showing relationship between response time (s) and percent correct (%) for the focus of expansion task. The black line denotes a negative correlation.

5.5 Discussion

These results highlight that V3A must be necessary for interpreting FOE position as application of TMS to V3A successfully disrupts performance on this task. In contrast, for the same task, application of TMS to TO-1 and TO-2 produces no significant change in performance. This is in agreement with previous literature indicating that signal increases in V3A modulate according to changes in position of the FOE (Koyama et al., 2005), and provides direct evidence of a causal relationship between neural activity in V3A and the signaling of FOE position. It is also consistent with case study evidence of a patient with damage to TO-1 and TO-2 whose ability to determine FOE

position remained preserved (Beardsley and Vaina, 2005a). This indicated the patient had function of a separate area that analysed FOE position, and the results presented here demonstrate that the patient must have been using V3A to do this task.

However this data is in direct dispute with TMS evidence from Harvey et al., (2010) who showed no effect of applying TMS to V3A on perception of FOE for rotational or radial motion in the majority of subjects (n=4). The direct discrepancy between the two experiments is likely explained by the variations in technique and localisation of the TMS. In the 2010 paper the TMS target site for V3A was an extrastriate dorso-medial region termed "V3A" that the authors discussed could potentially correspond to V2/V3 in some subjects. This experiment identified V3A using phosphene production and correlating the position of the phosphene with the known hemifield representation within V3A. Following application of TMS, the exact TMS co-ordinates were compared against an acquired retinotopic map and the correspondence with V3A is not sufficient enough to extract conclusions regarding specific functionality of this area (see Figure 5.9). In the present study, however, we directly targeted V3A by using retinotopic mapping paradigms to accurately identify it in all six individuals. This means that the data presented here is likely to be a good representation of functionality of V3A and not confounded by stimulation of V2 or V3.

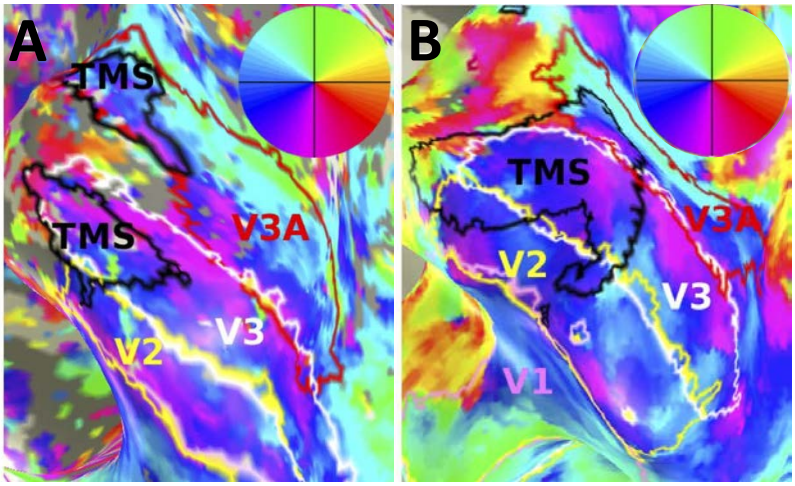

Figure 5.9. Figure showing correspondence of TMS target sites (black outline) with area V3A (red outline) of two subjects (A = AC; B = BH). (Adapted from Harvey et al., 2010).

The data reported here also highlights a difference between areas MSTd in non-human primates and TO-2 in humans, as previous research has shown that 90% of 245 recorded neurons within MSTd produced a differential response when the FOE was moved (Duffy and Wurtz, 1995). This indicates that if human TO-2 was homologous to MSTd then application of TMS to TO-2 should have disrupted subjects' performance on the FOE task in this experiment. However as this was not found to be the case then this could either be interpreted as showing that TO-2 is not homologous to MSTd, or as discussed in Chapter 4.1, there are fewer similarities across species than previously proposed.

Crucially, these results also highlighted a significant interaction between task and TMS condition (Figure 5.6). This shows that the areas involved in the processing of the two tasks are independent of one another. In other words, this experiment has identified a double dissociation between TO-2 and V3A for the perception of radially moving stimuli. This can be conceptualised as

TO-2 processing radial direction independent to area V3A processing the position of the FOE. In functional terms, this would suggest that if one of these areas was inhibited or damaged, the other area would still be functionally successful which proposes a parallel, as opposed to serial, processing pathway. This lack of dependence also further supports case study evidence of Beardsley and Vaina's (2005a) patient because despite damage to TO-2, she was still able to identify the position of the FOE by, presumably, using her functional V3A.

In a broader context, this data presents proposals as to how the human brain is able to perceive self-motion (heading/egomotion) information as we move through space. Expanding (radial) motion is naturally apparent when a person moves forwards through space and the focus of expansion corresponds to the direction in which the person is travelling. Appropriate interpretation of these combined cues is essential for successful navigation of the visual world. A previous fMR-adaptation experiment has shown that TO-1, TO-2 and V3A all show neural sensitivity to the location of an FOE, and these researchers propose that distinction of FOE positional information may begin in V3A (Cardin et al., 2012). This also highlights the importance of using causal evidence to reinforce neuroimaging data as the Cardin et al., (2012) experiment proposes TO-1 and TO-2 are also involved in identifying the position of the FOE, whereas this experiment has shown that although they may be involved, they are not necessary. Instead, it may be the case that TO-2 is not responsible for the analysis of self-motion, but rather an intermediate stage that contributes to the processing of self-motion (as proposed by Wall and Smith (2008)).

In terms of signal integration, the results outlined in this experiment seem to suggest that self-motion must be analysed within a cortical network of areas (incorporating TO-2 and V3A) that independently process elements of radial motion in order to determine whether we are moving forwards or backwards and in which direction (i.e. left, right). This proposes then that these signals must be combined in an area higher up the processing hierarchy. However this experiment does not reveal a complete explanation of self-motion processing as complex (spiral) motion and translational motion have also been shown to play a role in informing direction (Pitzalis et al., 2013c). Additionally, a crucial factor for self-motion not assessed here is knowledge of eye position and gaze relative to the FOE (Wall and Smith, 2008). Rather, this experiment has produced a better understanding of key areas involved in processing self-motion overall. It will be important in the future to investigate where in the hierarchy of visual areas these signals are combined following analysis in V3A and TO-2 in order to produce the complete perceptual information. Previous work has hypothesised that this could be achieved in area V6 (Pitzalis et al., 2010; Pitzalis et al., 2013a; Pitzalis et al., 2013b), as it has already been reported that V6 integrates depth and flow information to improve estimations of self-motion (Cardin and Smith, 2011). There is also evidence to suggest that area V6 receives some input from area V3A, as it exhibits a late visual evoked potential (VEP) which is thought to correspond to feedback from an extrastriate area like V3A (Pitzalis et al., 2013a). This corresponds well with findings reported here as we have shown that V3A is contributing to self-motion by determining the position of the FOE, and therefore it is a likely candidate to be feeding-forward to other areas involved

in this self-motion analysis. Future work to further investigate the relationship between V3A and V6, along with causally determining the function of V6 will likely reveal further evidence to support the involvement of TO-2 and V3A in the processing of self-motion.

Unlike TO-1 and TO-2 which are often compared to homologous areas in non-human primates (MT and MSTd, respectively (Saito et al., 1986)), V3A is not attributed such homology so easily. In non-human primates the area most likely to correspond both positionally and retinotopically to human V3A (mV3A) contains a large proportion of neurons that are not direction or speed selective (Gaska et al., 1988). This means that mV3A in macaque monkeys is not considered a motion-sensitive area and therefore this experiment has contributed to a wide range of literature highlighting this particular heterology between V3A across the species (Orban et al., 2003).

In order to be confident of the validity of these results, several analyses of secondary dependent variables were carried out to ascertain whether these there was any uncontrolled influence on the percent correct. These variables included: time taken to respond, response bias, and speed-accuracy trade-off. No significant differences were reported for response time or response bias which reassures that the data obtained for percent correct was likely not confounded by these measures. This also shows that response times and biases did not vary across TMS conditions. And importantly, response bias for V3A and TO-2 did not significantly diverge from zero (no bias) which means responses were not confounded by a particular perceptual bias. A significant divergence was reported for TO-1 in this task but application of

TMS to TO-1 was not found to disrupt performance so this result is not likely to have affected the final outcome of the data.

The speed-accuracy correlational analyses revealed that slow response times were moderately correlated with worse performance. This is the opposite to what would be expected following a speed-accuracy trade-off as it shows subjects perform better when responding quickly, which is likely a function of being confident in their decision. Hard trials result in slower response times which probably result from perceptual uncertainty.

In conclusion, area V3A must be necessary for adequate perception of the position of the FOE, and it processes this independently of area TO-2 which is responsible for interpreting the direction of the radial motion associated with the FOE. These areas are likely working independently within a cortical network of areas that contribute to heading (self-motion) perception.

Chapter 6
General Discussion and Future Work

6.1 Overview

Human visual cortex comprises a network of functionally specialised clusters of neurons; all of which are divided into different regions based on their function and representation of the visual world (Zeki et al., 1991). However the accuracy of the functional localisation within these identified divisions in the human brain is largely dependent on the quality of the design of the measurement and also the restrictions of the technology being used. For example, neuroimaging experiments are useful for allowing indications to be made regarding the involvement of visual areas relative to certain behavioural and cognitive tasks, however there are many limiting factors to consider: 1) the chosen voxel size restricts accuracy if it is too large to discriminate between smaller visual areas, 2) neuroimaging can only correlate neural activity with certain tasks, it cannot infer causality. To this end, techniques such as transcranial magnetic stimulation (TMS) are useful for providing causal evidence to support evidence from neuroimaging studies (Walsh and Cowey, 2000).

This thesis has focused on functionally defining the recently identified sub-divisions of hV5/MT+ (TO-1 and TO-2) by testing a selection of different motion paradigms that are known to successfully distinguish between potentially homologous areas in non-human primates (Saito et al., 1986; Duffy and Wurtz, 1991a; Duffy and Wurtz, 1991b). This functional division has been achieved across the experiments by using a combination of fMRI

localisation and targeted TMS methodologies. The following sections present the overall findings from this thesis and relate these results to the current literature and the implications they have for our understanding of function of the human brain.

6.2 Identification of hV5/MT+ Sub-Divisions

In order to accurately position the TMS coil, successful and reliable identification of TO-1 and TO-2 was required due to the individual differences between individuals in the anatomical location of visual areas (McKeefry et al., 2009). This was particularly crucial for this experiment as data from Sack et al., (2009) has shown that fMRI-guided TMS is the only reliable method of achieving a significant behavioural effect with less than nine subjects as described in this thesis (n = 6). The accurate localisation of TO-1 and TO-2 had reliably been accomplished in previous experiments utilising functional localisers designed to activate ipsilateral representations within TO-2 (Dukelow et al., 2001; Huk et al., 2002; Amano et al., 2009), and was successful in 13/14 hemispheres within this thesis using the same technique (see Chapter 2; Section 2.6). The potential issues with relying on functional (as opposed to retinotopic) localisation lie in that the functional localiser only identifies areas within hV5/MT+ that have ipsilateral representations. It is presumed that this corresponds to TO-2 because of the larger receptive field size of neurons within this area. However, researchers have previously successfully retinotopically mapped this region and found that the cluster of activations produced by this functional localiser produces good correspondence with the retinotopic representation presumed to be TO-2

(Amano et al., 2009). This allows us to be confident in our localisation of this area. We also converted our Native co-ordinates for TO-1 and TO-2 into Talairach co-ordinates in order to compare between studies and found that they were very similar to those reported by Dukelow et al., (2001) and Kolster et al., (2010) (see Chapter 2; Section 2.5.3; Table 2.1).

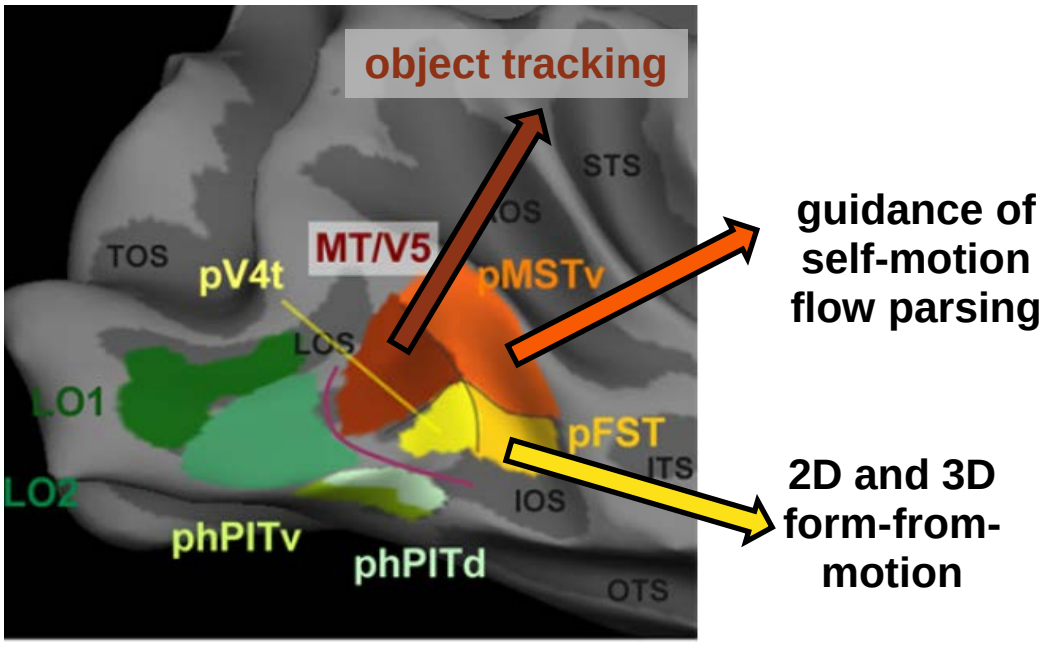

Figure 6.1. Inflated right hemisphere showing location and position of proposed sub-divisions of hV5/MT+ (MT/V5 corresponds to TO-1 and pMSTv corresponds to TO-2). The figure also demonstrates potential origins of separate motion processing pathways with their proposed functions adapted from non-human primate literature. (Adapted from Kolster et al., 2010).

It is important to note, however, that in non-human primates the MT+ complex contains up to five sub-divisions rather than two (Nelissen et al., 2006; Orban et al., 2003; Kolster et al., 2009), and one lab has tentatively managed to topographically identify five sub-divisions (pMT, pMSTd, pMSTv, pFST, pV4t; p = putative; see Figure 6.1) within human cortex by using an

alternative neuroimaging approach that combined population receptive field (pRF) estimates with a modified retinotopic mapping stimulus (Kolster et al., 2010). Indeed, similar to the non-human primate cortex, these sub-divisions have been associated with analysis of differing aspects of visual motion processing including analysis of two- and three-dimensional form from motion (Xiao et al., 1997; Kolster et al., 2009; Kolster et al., 2010). The work presented in this thesis was unable to find evidence of separate ventral retinotopic maps corresponding to FST or V4t, however, as this thesis was not specifically investigating the existence of these further sub-divisions, we cannot conclude that they do not exist. Instead, as already discussed, it should be noted that the Talairach co-ordinates for TO-1 and TO-2 align closely with that of pMT and pMSTv described by Kolster et al. (2010), thereby suggesting that the sub-divisions presented here are likely separate to any potential inferior sub-divisions. It should also be noted that Kolster et al. (2010) aligned their sub-divisions with homologous non-human primate cortical areas based on retinotopic criteria alone. They did not assess the functionality of this area with respect to optic flow stimuli which has previously successfully differentiated between functions of MSTd and MSTl/v in non-human primates (Desimone and Ungerleider, 1986; Komatsu and Wurtz, 1988; Tanaka and Saito, 1989), and provided some implications for TO-1 and TO-2 in humans (Smith et al., 2006; Wall et al., 2008). Therefore a link between TO-2 and MSTd cannot be conclusively ruled out until a clearer understanding of the functional properties of human pMSTv has been reached.

6.3 Functional Dissociation of TO-1 and TO-2

The main aim of this thesis was to provide causal evidence of a functional distinction between TO-1 and TO-2 in the human brain. Previous neuroimaging studies had highlighted potential differences between these areas when viewing expanding/contracting motion (Smith et al., 2006; Wall et al., 2008), so the experimental tasks for the fMRI-guided TMS were designed around this type of stimuli.

The results reported in this thesis provide novel discoveries regarding the function of TO-1 and TO-2 because they provide the first causal evidence of a single dissociation between the two sites. It was discussed in Chapter 4.1 that area TO-1 is functionally responsible for processing translational (up/down) motion, whilst TO-2 is responsible for both processing translational and radial (expanding/contracting) motion. This contributes to the findings from previous literature showing that TO-1 and TO-2 demonstrate slightly different BOLD responses to these types of motion (Smith et al., 2006; Wall et al., 2008). These results also support the notion that anterior area TO-2 may be processing radial (expanding) motion due to possessing a role in the processing of self-motion/heading (Cardin et al., 2012; Pitzalis et al., 2013c). This suggests an increasing level of complexity as sub-divisions within hV5/MT+ become more anterior, which corresponds to that of non-human primates (Duffy and Wurtz, 1991a; Duffy and Wurtz, 1991b; Duffy and Wurtz, 1997). These results also support the notion of a serial framework for analysis of translational motion, as application of TMS to both TO-1 and TO-2 affected performance on translational motion tasks. This

would imply that either TO-1 is feeding the signal forward to TO-2 for further analysis, or that TO-2 is feeding the signal back to TO-1 following analysis. Further investigations into the temporal characteristics of these areas by investigating the impact of varying onsets of TMS pulses relative to stimulus onset may provide further explanation as to which of these two possibilities is most likely.

In keeping with a comparison to potentially homologous areas within non-human primate cortex, the results provided in this thesis seem to suggest that TO-2 produces functionality similar to that of MSTd, due to the observed selectivity to radial flow patterns (Saito et al., 1986; Tanaka and Saito, 1989; Lagae et al., 1994; Duffy, 1998; Duffy and Wurtz, 1991a; Duffy and Wurtz, 1991b). However, this is directly at odds with previous proposals of homology between human TO-2 and non-human primate MSTl/v made on the basis of similarities between known RF characteristics of the two areas (Amano et al., 2009). Nevertheless, functionally, MSTl/v is responsible for maintenance of pursuit eye movements and tracking (Komatsu and Wurtz, 1988; Dursteler and Wurtz, 1988; Komatsu and Wurtz, 1989), and not typically responsible for processing of optic flow (Eifuku and Wurtz, 1998), which is in direct disagreement with the data presented in this thesis. These conflicting RF and functional properties across species highlight the difficulties in establishing clear homologies between the sub-divisions of human and non-human primate V5/MT+. Indeed, there is even evidence to suggest that finding homologies between sub-divisions of MT+ across different species of monkey is not always possible so perhaps expecting to find pure homology is unlikely (Rosa et al., 1993).

6.4 Double Dissociation of TO-2 and V3A

An important result obtained from the focus of expansion (FOE) experiment described in Chapter 5 was the double dissociation observed between TO-2 and V3A relating to the processing of radial direction and FOE. This result allowed us to conclude that these areas must be processing these elements of visual motion independently of one another, and therefore it is likely that they form separate streams of a parallel processing pathway. Given the characteristics of the dissociation, this pathway is proposed to be linked to the analysis of self-motion (motion elicited by movement of the individual). This coincides with research investigating temporal characteristics of visual motion areas when viewing self-motion, as they found that initial VEPs were almost identical in V6 and hV5/MT+, but that the VEP recorded from V6 received a second signal, thought to correspond to feedback from extra-striate areas (Pitzalis et al., 2013a). This was proposed to originate in V3A, but it is possible that this second VEP could result from feedback originating from both V3A and TO-2, as V6 has often been correlated with analysis of self-motion so it would follow that signals from self-motion processing would culminate in this region (Pitzalis et al., 2010; Pitzalis et al., 2013b; Pitzalis et al., 2013c).

6.5 Further Directions

Further work will need to investigate the role of TO-2 in the processing of radial motion. In the experiments described in Chapter 4.1, the stimuli were designed to expand and contract around the centre of the presentation

aperture because ‘true’ radial motion (originating at fixation) resembled translational motion moving from left to right (see Figure 6.2). This meant the central radial stimuli used were appropriate for the task, and allowed appropriate comparisons to be made between the direction discrimination task and the FOE task, however it will be important to compare results to a radial task in which the centre of the motion is at fixation. This may require an aperture that is longer vertically in order to reduce the perception of translational motion, but this is something that can be assessed in future experiments.

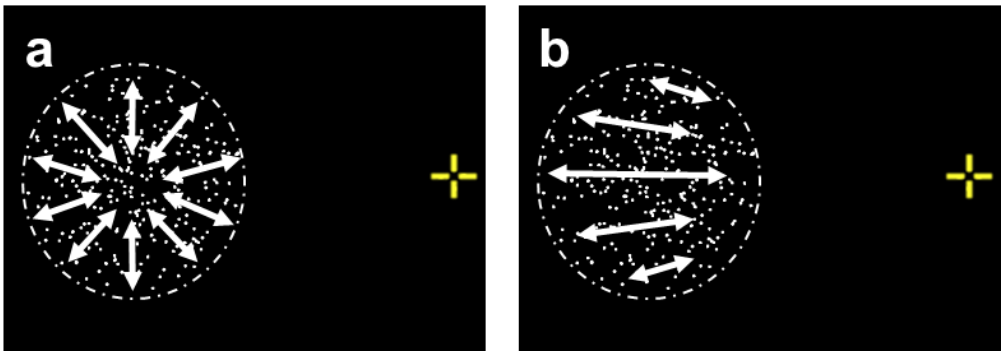

Figure 6.2. Diagram outlining the difference between presenting radial motion with the FOE at the centre of the aperture (a) versus the fixation (b).

Secondly, it will be important to confirm the existence of pFST and pV4t in human cortex by collecting fMRI data that utilises: 1) adapted retinotopic mapping protocols and pRF measurements (Kolster et al., 2010), and 2) multi-variate pattern analysis (MVPA). Both techniques will allow further parcellation of hV5/MT+ and associated pathways by assessing the receptive field characteristics of populations of neurons (pRF mapping) and assessing information contained within distributed patterns of neural activity to make

inferences regarding functionality of these areas (MVPA). MVPA relies on the use of classifiers that attempt to capture relationships between spatial patterns of fMRI activity elicited by various types of visual stimuli (in this case, moving stimuli), and has previously been successful in demonstrating that different object categories are represented in a distributed manner across the ventral brain (Haxby et al., 2001). Once the MVPA analysis has informed us of the distributed functional roles of sub-divisions of hV5/MT+, it would then be possible to extend this analysis across the cortical motion network as a whole in order to ascertain whether it can also corroborate the existence of separate processing hierarchies and networks. This would provide complementary information as to how different visual areas interact in the perception of different types of moving stimulus.

Once these sub-divisions have successfully been identified using fMRI, then it will also be possible to target them for application of TMS. This would allow functional distinctions to be made based on causal evidence of differences between TO-1, TO-2, FST and V4t in the human brain. Given the functional similarities with comparable areas within the non-human primate cortex, the most efficient way to distinguish between these areas would be to test for characteristics of processing that are already correlated with neuronal activity within FST and V4t in monkeys; including extracting form-from-motion and complex motion (Nelissen et al., 2006). Psychophysical procedures could include tasks involving motion-defined boundaries such as that shown in Figure 6.3a. Subjects could identify the location of the boundary with coherent dots at individual threshold levels. A second potential experiment could involve perception of a motion-defined shape such as Figure 6.3c. This

task could involve the discrimination of two shapes, with motion moving in an opposite direction within the shape. Again, this would be at threshold level so as to measure percent correct. Two further tasks hoping to categorise further distinctions between TO-1 and TO-2, could use complex stimuli designed to elicit a response from TO-2. The Figures 6.3b and 6.3d indicate potential complex stimuli such as differing planes of motion defined by dot speed (subjects could indicate whether the top of the aperture is tiled away from or towards the subject), and spiral motion (characterised by combination of radial and rotational motion; subjects would be required to indicate direction of spiral). If application of TMS to any of these areas produced a behavioural deficit then it would be possible to conclude that perception of that task relies on neural activity within the targeted area.

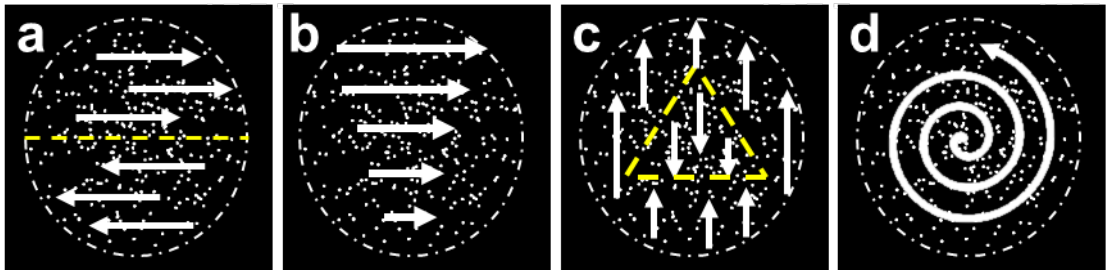

Figure 6.3. Diagram outlining types of motion to be tested in future psychophysical experiments. Kinetic boundaries defining shapes from motion (a), speed gradients (perceived as planes of motion tilted in depth; arrows of different lengths denote differences in speed) (b), 2-Dimensional form defined by motion (c), and spiral motion (d).

It will also be crucial in future experiments to assess the relationship between V3A, TO-2 and V6 in the processing of self-motion. This thesis has reported that TO-2 is responsible for processing radial direction whilst V3A is responsible for processing focus of expansion (FOE), but a significant

interaction shows that these areas are processing these independently of one another. This then means that the signals must be integrated further up the visual hierarchy and it is proposed that this may occur in area V6 (Pitzalis et al., 2013a). The only potentially limiting factor of investigating the function of V6 is that it is typically located medially along the parieto-occipital sulcus (Pitzalis et al., 2010). This may cause difficulties in application of TMS to this area, as if it is occluded by another visual area or if it is located too deep within the cortical cavity for the TMS pulse to reach, it may not be possible to produce measurable effects. Providing the technique is successful however, it will be important to assess the role of V6 in the processing of flow parsing (Pitzalis et al., 2006; Cardin et al., 2012). Flow parsing is defined as the separation of self-generated (observer-generated) motion signals from those generated by the motion of real objects within the visual environment (Figure 6.4).

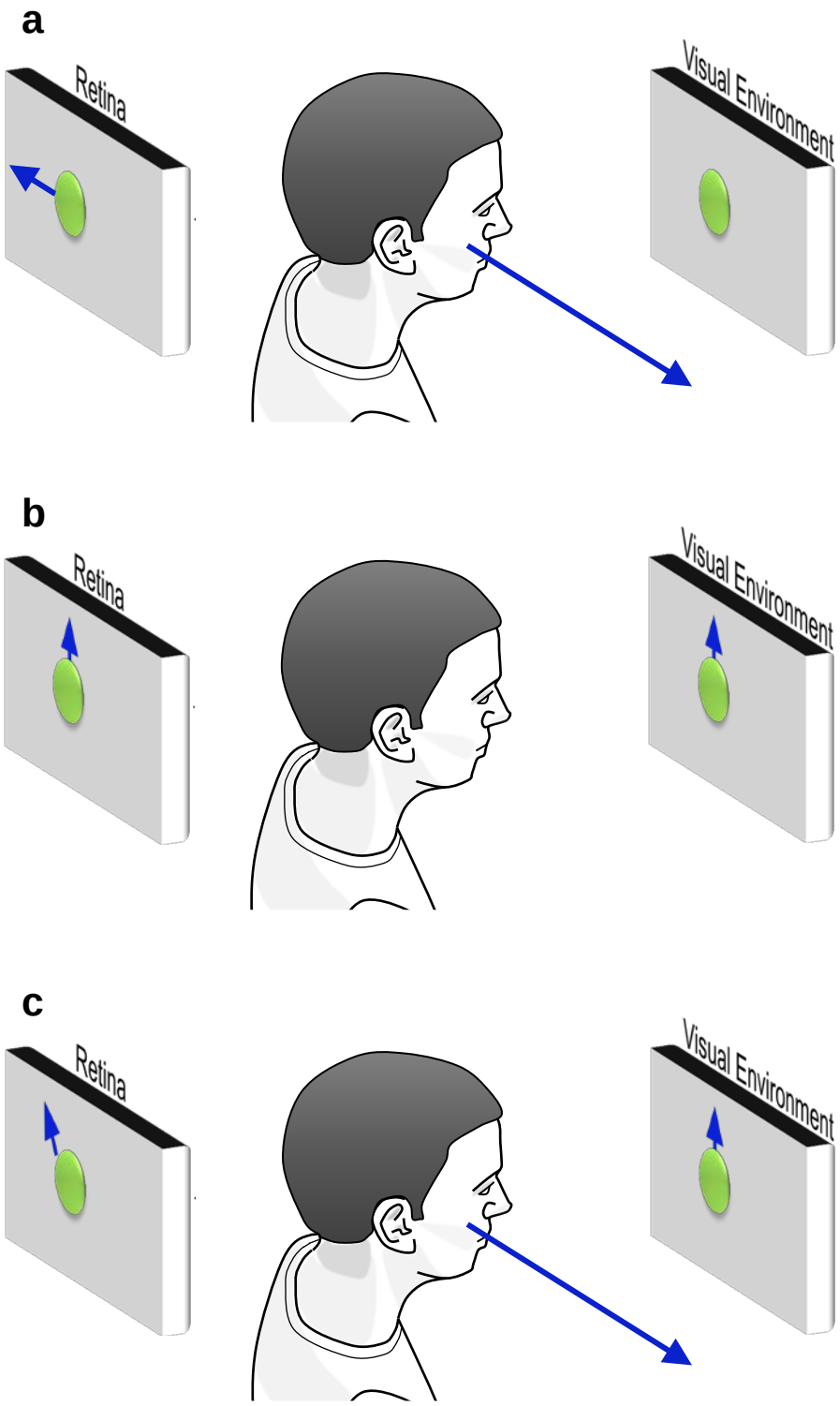

Figure 6.4. Schematic of flow parsing error made when observer moves. Motion is perceived when both a) the environment remains stationary but the observer moves, and b) when the observer remains stationary but the object moves. If both the observer and the object move then the observed motion of the object is combined.

One way to test whether area V6 is involved in the processing could be to design an experiment that utilises the relative tilt aftereffect (Figure 6.5). This is the idea that when viewing expanding optic flow stimuli in one hemifield, a dot moving vertically downward in the opposite hemifield is perceived to be tilted towards the expanding motion (Warren and Rushton, 2009; Warren et al., 2012). It would be hypothesised that if area V6 was processing flow parsing, then the extent of the tilt aftereffect would be reduced if TMS was applied. If found to be the case; this would provide evidence of causal dependence between neural activity in V6 and flow parsing mechanisms.

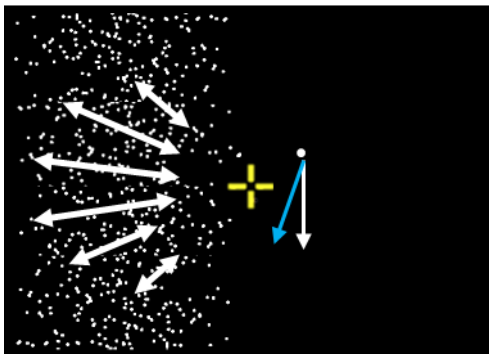

Figure 6.5. Schematic of relative tilt aftereffect when viewing expanding motion in left hemifield. White arrows represent real direction. The blue arrow represents perceived direction.

6.6 Conclusions

Using fMRI-guided TMS, this thesis has confirmed existence of two sub-divisions of hV5/MT+, TO-1 and TO-2, and then proceeded to functionally parcellate them based on direction discrimination tasks. The evidence shows that these regions both contribute to the processing of translating motion whilst only TO-2 is responsible for processing radial motion. This thesis has also determined a double dissociation between TO-2 and V3A in the

processing of radial direction and position of FOE, showing that independent processing of these features occurs across the two areas.

It has also contributed to the literature assessing cortical homology between non-human primates and humans by demonstrating that although there are similarities between hV5/MT+ and MT+, there also appear to be marked functional and receptive field characteristic differences, therefore suggesting that pure homology is not likely.

Taken as a whole, this thesis has provided evidence of both parallel and serial processing pathways within motion processing in the human brain, and has provided hypotheses for the processing of combinations of these features of motion (e.g. self-motion).

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Appendix 1 – Participant Consent Form

CONSENT FORM

Project Title: *The Functional Sub-Divisions of Human Motion Sensitive Visual Cortex. Section 1: TO-1 and TO-2*

Researchers: *Samantha Strong (s.l.strong@student.bradford.ac.uk)*
Dr. Declan McKeefry (d.mckeefry@bradford.ac.uk)
Prof. Tony Morland (a.morland@psych.york.ac.uk)

Please Circle

1. I have read the information sheet entitled "The Functional Sub-Divisions of the Human Motion Sensitive Visual Cortex: An fMRI Guided TMS Study: Participant information sheet". **YES / NO**

2. I have had the chance to discuss the study and to ask questions, and I have had satisfactory answers to all of my questions. **YES / NO**

3. I understand that I am free to withdraw from the study, at any time and without having to give a reason. **YES / NO**

4. Do you agree to take part in the study? **YES / NO**

5. I understand that I can discuss the study with a researcher at any time. **YES / NO**

6. I know that the information that I will provide will be kept strictly confidential. When the results are published no individual person will be identified in any way without that person's written agreement. I agree that other researchers may access the data from this study for use in research and teaching. Those researchers will require approval of the Research Ethics Committee of the YNiC and the University of Bradford and will be allowed access to my data in anonymous form only. **YES / NO**

7. If I have any questions or concerns about the research, I know I can contact any of the researchers listed at the top of the page. **YES / NO**

Name of Participant	Date	Signature

Name of Researcher	Date	Signature

Appendix 2 – Participant Information Sheet

York NeuroImaging Centre
THE UNIVERSITY of York

Participant Information

The Functional Sub-Divisions of the Human Motion Sensitive Visual Cortex:
An fMRI Guided TMS Study

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

This sheet tells you the purpose of this study and what will happen to you if you take part. Please also read the information leaflets on magnetic stimulation (TMS) and MRI (magnetic resonance imaging). Both of these techniques are employed in this study.

Please ask if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

The perception of motion is known to be the result of neural activity across a large number of regions in the brain. These regions include visual areas known as V3, V3A, V3B and an important area known as hV5/MT+. We aim to study how these brain regions contribute in their own particular way to the perception of moving stimuli. In order to do this we will be using a technique known as fMRI-guided Transcranial Magnetic Stimulation (TMS).

Why have I been chosen?

We are seeking to recruit 12 healthy adults for this experiment. Participants are right-handed people between 18-50 years old, with normal or corrected-to-normal vision and hearing and no known neurological or psychiatric conditions.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and will be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason.

What will happen to me if I take part?

The study will involve attending the York Neuroimaging Centre several times. The number of sessions can vary but is normally between two and four. Each session will last for approximately one hour.

Before taking part, you will be asked to fill out some questionnaires. These will ask you about your medical history, about drugs you might be taking and

whether you might be pregnant. This information is important to ensure your safety. Your answers will be kept strictly confidential.

During session one, we will obtain a structural MRI scan, retinotopic mapping and two functional localiser scans. This will involve lying still for in the MRI scanner for up to an hour so that an image of your brain can be acquired and functional data can be used to guide the TMS coil to specific stimulation sites. Usually, this is the only fMRI scanning session required for the study, but under some circumstances we may need to follow up with additional measurements and this could require another fMRI session.

During subsequent sessions, we will use magnetic stimulation to investigate which brain regions process motion coherence. In magnetic stimulation, the researcher holds a magnetic coil against the scalp. The magnetic pulses pass through the skull and make the brain cells underneath the coil fire; this causes temporary disruption to normal brain function. During magnetic stimulation the researcher will ask you to perform a discrimination task. Normally, we will split this component of the study into multiple sessions to reduce the participant's commitment of time to a particular day and to reduce fatigue. We aim to perform all elements of the study in no longer than 4 hours. However, we would normally hope to perform each of the elements in a shorter time.

Please read the attached information leaflet on MRI which provides further details about this technique. Additional information regarding TMS can be found at the York TMS: LAB website
www.york.ac.uk/res/tms/TMS_Lab/Welcome.html

Are there any risks of taking part?

Magnetic stimulation (TMS) is a well-established technique used safely in many laboratories and hospitals around the world. However, in susceptible individuals, it can increase the risk of having an epileptic seizure. For this reason, you will not be able to take part in this study if you have a family or personal history of epilepsy, if you are taking certain medications or if you have a neurological or psychiatric condition.

Magnetic stimulation and MRI both involve strong magnetic fields. Consequently, you will not be able to take part if you have a heart pacemaker, cochlear implant or other biomedical implants containing ferrous metal (including dental braces), or if you could have metal in your eye. Pregnant women cannot take part. You must not take any metal objects into the scanner or magnetic stimulation room.

The magnetic stimulation produces a tapping sensation on the scalp and sometimes causes eye blinks and facial twitches that can be mildly uncomfortable. Please tell the experimenter if you experience any problems as the stimulation can be adjusted to minimise these effects.

For the MRI scan, you will need to lie very still for up to an hour. The scanner can induce feelings of claustrophobia in susceptible people and is very noisy (you will wear ear plugs). People also sometimes experience disequilibrium and/or mild nausea if they move quickly in the scanner room.

What will happen if I don't want to carry on with the study?

If you feel uncomfortable about any aspect of the study, please let the researcher know straight away. S/he will be discuss your concerns with you and may be able to help. You are free to withdraw from the study at any time. If you decide to withdraw, please tell the researcher.

We will not normally be able to use behavioural data from participants who withdraw from the study. Data that has already been collected will be destroyed if it cannot be used and will always be deleted at the participant's request.

What if there is a problem?

If you have a concern about any aspect of this study, please speak to the researchers who will do their best to answer your questions. You can contact Dr. Declan McKeefry, who is leading this study (d.mckeefry@bradford.ac.uk). If you remain unhappy and wish to complain formally, you can do this through the complaints procedure of the University of York.

The York Neuroimaging Centre takes pride and care in ensuring that no harm, or risk of harm, occurs to participants in research. In the event that something does go wrong and you are harmed during the research study and this is due to someone's negligence, then you may have grounds for a legal action for compensation against The University of York.

Will my taking part in the study be kept confidential?

Any information that you give us and all of the measurements that we collect will be confidential. No names will be used when the research is written up. The information will be stored in anonymous computer files and in locked filing cabinets. Names and addresses will be stored separately from other data.

We will use your data in this study and may combine your data with data that we gather in future studies. We will keep your data for 10 years and will then destroy it securely. We will comply with the terms of the Data Protection Act 1988.

The contact details of participants will be accessible to Prof. Tony Morland and his research team but will not be passed to anyone else. In addition, staff of the York Neuroimaging Centre have privileged access to the computer systems and can link the names of participants with their data. Those people are under a professional obligation not to abuse this privilege. With the approval of the Research Ethics Committee of the York Neuroimaging Centre, other researchers may be allowed access to the data for use in research and teaching but in anonymous form only.

We are not qualified to interpret brain images clinically. If we suspect that an image of your brain reveals a possible problem, we will inform your GP (family doctor) who may then contact you and advise you. If you do not want us to do this, then you should not agree to take part in the study.

What will happen to the results of the study?

The results will be written up in scientific journals and talked about at conferences. It will not normally be possible to give feedback about performance to individual participants because the data are taken away and analysed at a later date. However, the lead researcher, Dr. Declan McKeefry, will be happy to provide a summary of the results when they become available.

Who has reviewed the study?

This study was given a favourable ethical opinion by the Research Ethics Committee of the York Neuroimaging Centre and by the Biomedical, Natural, Physical and Health Sciences Research Ethics Panel at the University of Bradford on 30th January 2013.

Who is organising and funding the research?

This study is being led by Dr. Declan McKeefry who is a Reader in Vision Science at the University of Bradford.

Further information

If you are concerned about safety and how the MRI procedure takes place, you can read medical websites (http://www.nhsdirect.nhs.uk/articles/article.aspx?articleId=556; http://www.ehealthmd.com/library/mri/MRI_risks.html).

If you are concerned about safety and how the TMS procedure takes place, you can read the following journal article: *Wasserman EM. (1998). Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Transcranial Magnetic Stimulation. Electroenceph Clin Neurophysiol 108,1-16.*

We hope the information in this document helps to prepare you for our study. If there is any information you want to know, please do not hesitate to e-mail us or discuss it on the day of your scan. After the scan, we will inform you of the nature and purpose of the experiment, and you will have a chance to learn something from our research.

Thank you very much for your time!

Declan McKeefry
University of Bradford
d.mckeefry@bradford.ac.uk

Samantha Strong
University of Bradford
s.l.strong@student.bradford.ac.uk

Appendix 3 – TMS Safety Screening Form

THE UNIVERSITY of York

York Neuroimaging Centre

The Biocentre, York Science Park, Heslington, York, YO10 5DG

Tel. 01904 435329, Fax 01904 435356

Confidential

TMS Safety Screening Form

If you agree to take part in this study, please answer the following questions. It is essential that you answer truthfully. The information you provide is for screening purposes only and will be kept completely confidential.

1. Have you ever suffered from any neurological or psychiatric conditions?

   YES/NO If YES please give details (nature of condition, duration, current medication, etc).

2. Have you ever suffered from epilepsy, febrile convulsions in infancy, had a fit or seizure or recurrent fainting spells?

   YES/NO

3. Does anyone in your immediate or distant family suffer from epilepsy?

   YES/NO If YES please state your relationship to the affected family member.

4. Have you ever had an operation on your head or spine (including eye surgery)?

   YES/NO If YES please give details.

5. Do you currently have any of the following fitted to your body?

   YES/NO Heart pacemaker
   Cochlear (ear) implant
   Medication pump
   Surgical clips
   Any other biomechanical implant

6. Have you ever had an injury to your eye involving metal fragments?

   YES/NO

7. Are you currently taking any unprescribed or prescribed medication?

   YES/NO If YES please give details.

8. Are you currently undergoing anti-malarial treatment?

YES/NO

9. Have you drunk more than 3 units of alcohol in the last 24 hours?

YES/NO

10. Have you already drunk alcohol today?

YES/NO

11. Have you had more than one cup of coffee, or other sources of caffeine, in the last hour?

YES/NO

12. Have you used recreational drugs in the last 24 hours?

YES/NO

13. Did you have very little sleep last night?

YES/NO

14. Have you already participated in a TMS experiment today?

YES/NO

15. Are you or could you be pregnant?

YES/NO

Participant details:

16. Are you left or right handed?

Left/Right

17. Date of birth

___/___/___

I understand that the above questions check for serious risk factors.

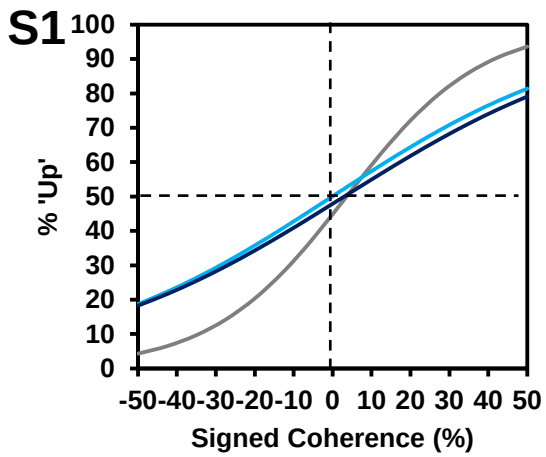
I CONFIRM THAT I HAVE READ, UNDERSTOOD AND CORRECTLY ANSWERED THE ABOVE QUESTIONS

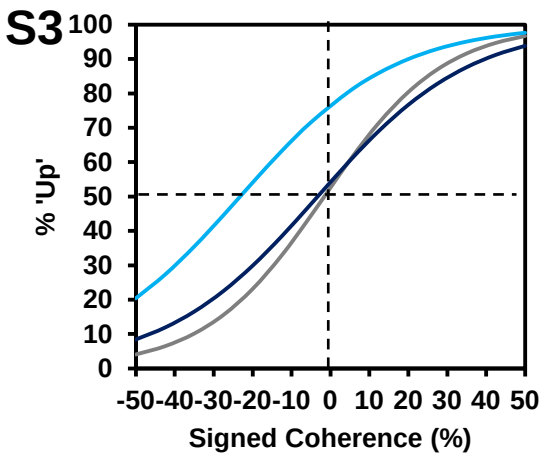
IN CASE OF ANY DOUBT, please inform the investigator before signing this form.

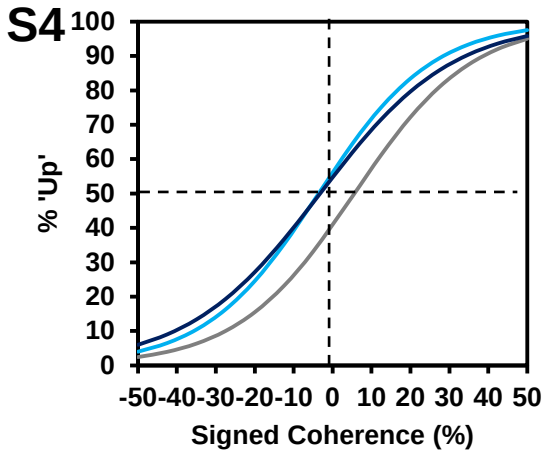
Participant's Name ……..……. Signature ……
Date ……..

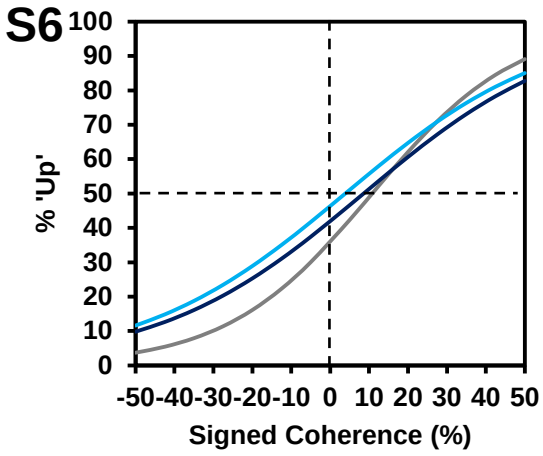
Researcher's Name ……..…… Signature ……
Date ……..

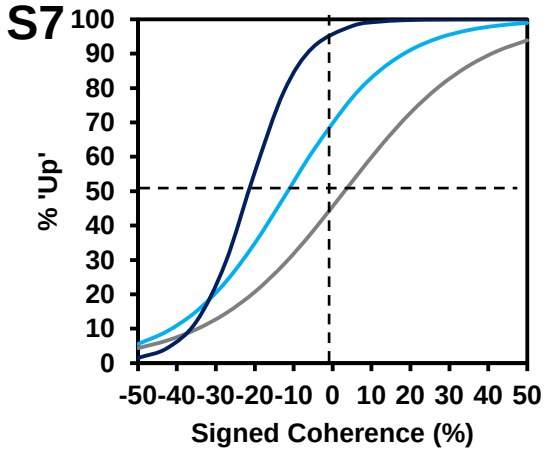
Appendix 4 – Individual Data for Translational Motion Task











Appendix 5 – Individual Data for Radial Motion Task